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PROTOZOOLOGY - ORIGINAL PAPER

Cecal coccidiosis in turkeys: Comparative biology of *Eimeria* species in the lower intestinal tract of turkeys using genetically typed, single oocyst–derived lines

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Abstract

Differentiating the *Eimeria* species causing cecal coccidiosis in turkeys is challenging. To obtain benchmark biological data for *Eimeria gallopavonis* Hawkins 1952 and *Eimeria meleagridis* Tyzzer 1929 and to support the stability of the species concept for each, genetically typed, single oocyst–derived lines of *E. gallopavonis* Weybridge strain and *E. meleagridis* USAR97-01 were used to redescribe the biological, pathological, and morphological features of these parasites. Oocysts of *E. meleagridis* and *E. gallopavonis* overlap in dimensions, but oocysts of the former have a single polar granule compared with multiple in the latter. Mature first-generation meronts of *E. gallopavonis* were observed histologically as early as 48 h post-inoculation alongside the villi in jejunum (before and after Meckel's diverticulum), ileum, cecal neck and rectum, but not cecal pouches. Three asexual cycles were observed suggesting that early workers apparently overlooked one asexual cycle. Examination of endogenous development of a culture labeled "*Eimeria adenoeides* Weybridge strain" suggested that this strain (found in a number of publications as a large oocyst strain of "*Eimeria adenoeides*") matched the species description of *E. gallopavonis* and so has been renamed herein. Macroscopic lesions induced by *E. gallopavonis* consisted of caseous material distally from posterior of the yolk stalk through the remaining intestinal tract, excluding the cecal pouches. For *E. meleagridis*, only the first asexual generation was observed outside of the cecal pouches within the jejunum around the yolk stalk. Second- and 3rd-generation asexual stages developed almost exclusively in the cecal pouches (but not cecal necks). Macroscopic lesions described for *E. meleagridis* were similar to those of *E. adenoeides*. Marked corrugation of the cecal serosal surface was observed. Cecal pouches contained creamy colored, caseous material varying from loose material to granular. Distinguishing features of the *Eimeria* species infecting the lower part of the small intestine are summarized in the present study, and new type specimens were designated for *E. gallopavonis* and *E. meleagridis* to provide a stable reference for future work with these parasites.

Keywords *Eimeria gallopavonis* · *Eimeria meleagridis* · *Eimeria adenoeides* · Coccidiosis · Turkey *Eimeria* · Life cycle · Endogenous development · Nuclear 18S rDNA · Mitochondrial cytochrome *c* oxidase subunit I · Taxonomy

Introduction

Coccidiosis is a parasitic disease caused by intracellular protozoan parasites belonging to the genus *Eimeria*. These parasites invade the enterocytes of the intestinal tract and reproduce to complete their life cycle that can damage the intestinal mucosa, sometimes seriously. After the end of the life cycle, the parasites are excreted from the host in an environmentally resistant form (the oocyst). Seven described *Eimeria* species are considered responsible for coccidial infections in turkeys. Three of them, *Eimeria adenoeides* Moore and Brown 1951, *Eimeria meleagridis* Tyzzer 1929 (see Williams 2010), and *Eimeria gallopavonis* Hawkins 1952 infect the lower part of the intestine (ileum, cecum, and rectum). These species are

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considered to be pathogenic and have been linked to macroscopic pathological lesions observed in clinical coccidiosis outbreaks in turkey flocks (Moore and Brown 1951; Hawkins 1952; Clarkson 1958; Rampin et al. 2006). The lesions described for these *Eimeria* species have been reported to be similar; sloughing of the epithelium, edema of the intestinal wall, and characteristic white caseous material have been described mainly in the lower part of the intestine starting in the ileum and continuing through to the rectum with the characteristic caseous plugs filling the two cecal pouches (e.g., Moore and Brown 1951; Hawkins 1952; Clarkson 1959). The original morphometric and biological species descriptions of these parasites (i.e., Tyzzer 1929; Moore and Brown 1951; Hawkins 1952) were limited and incomplete by current standards; using data solely from these original species descriptions, the three species cannot be differentiated using oocyst morphometrics or pathological lesions in the intestinal tract of infected birds (Clarkson 1960). The result was confusing, and sometimes contradictory, data in later studies on these protozoan parasites; for example, McDougald and Fitz-Coy (2003) could not satisfactorily identify isolates from field samples based on established species descriptions. Linking a field outbreak with a particular *Eimeria* species infecting turkeys is especially difficult for these three cecal species (Rampin et al. 2006). Recent studies describing newly isolated strains of lower intestinal *Eimeria* species infecting turkeys have provided information on life cycle stages that differ in some aspects from the original descriptions (Matsler and Chapman 2006; Chapman 2008; El-Sherry et al. 2014a, 2014b). Another study provided information for the first time on two different strains of *E. adenoeides* with remarkable morphometric variation (Poplstein and Vrba 2011). The loss of all of the original strains used for these species descriptions makes it impossible to compare any new isolate with the type strains to address this confusion. At least some of the confusion in the literature could be explained by the presence of multiple species within putatively single-species isolates used for species descriptions and subsequent biological characterizations (e.g., Moore and Brown 1951; Clarkson 1958). Even in more recent attempts to study these species using molecular techniques and phylogenetic analyses, it proved impossible to provide a precise identification for all species because the sequences were not derived from single-oocyst isolates and field samples usually contain more than one species (Miska et al. 2010). The identification in such cases was made using classical morphometric criteria that are known to overlap considerably (Cook et al. 2010; El-Sherry et al. 2015).

The present work is part of a series of studies with the goal of isolating, purifying, and redescribing *Eimeria* species infecting turkeys using linked molecular and biological identification. The newly characterized single-oocyst lines derived for each species helped in solving some contradicting information regarding these species. For example, studying multiple single oocyst–derived lines of *Eimeria meleagrimitis* revealed the presence of divergent, paralogous 18S rDNA copies within the nuclear genome (El-Sherry et al. 2013) that were previously interpreted as two strains of *E. meleagrimitis* by Cook et al. (2010). The new molecular and biological data obtained clarified that the KCH and KR strains of *E. adenoeides* reported by Poplstein and Vrba (2011) represent two distinct species, *E. adenoeides* and *E. meleagridis*, respectively (El-Sherry et al. 2015; present study). Analysis of the Weybridge strain of *E. adenoeides*, which has long been used as reference strain in various studies conducted on the pathogenicity of *E. adenoeides* (e.g., Hein 1969; Joyner and Norton 1972), suggests that this coccidium more closely fits the species description of *E. gallopavonis* (El-Sherry et al. 2015; present study). Most recently, *E. adenoeides*, *E. meleagridis*, and *E. gallopavonis* were characterized morphologically and genetically at both the mt COI and nu 18S rDNA loci to provide sequence-based, reference genotypes (Ogedengbe et al. 2014; El-Sherry et al. 2015).

In the present work, we provide detailed biological descriptions for single oocyst–derived lines of *E. gallopavonis* Weybridge strain and *E. meleagridis* USAR97-01 strain including designation of new type specimens for each species. These biological redescriptions of *E. gallopavonis* and *E. meleagridis* are linked to sequence-based genotyping at nuclear (18S rDNA) and mitochondrial (COI) genetic loci. These defined biological and genetic features are then compared with similar data on *E. adenoeides* Guelph strain (El-Sherry et al. 2014b) to differentiate among the three *Eimeria* species that infect the lower intestinal tract of turkeys.

Materials and methods

Origins and derivation of single oocyst–derived lines of *E. gallopavonis* (Weybridge strain) and *E. meleagridis* (USAR97-01).

Eimeria gallopavonis (Weybridge strain). This strain was kindly provided by H. D. Chapman on July 22, 2011 under the name of "Weybridge" strain of *E. adenoeides*; the strain was isolated in 1952 by S. F. M. Davies at the Central Veterinary Laboratory (CVL), Weybridge, UK. Oocysts were obtained from poults submitted for diagnosis following an outbreak of coccidiosis. This "Weybridge strain of *E. adenoeides"* was used subsequently in a number of studies (see Hein 1969; Joyner and Norton 1972). Molecular genotyping and oocyst morphometrics studies (El-Sherry et al. 2015), as well as the present biological examination, have clarified that the characters of this strain agree with those identified by Hawkins (1952) for *E. gallopavonis.*

Eimeria meleagridis (USAR97-01 strain). This strain of *E. meleagridis* was obtained in 1997 from the ceca of a turkey from a commercial farm in Northwest Arkansas and purified by Dr. H. D. Chapman (Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA). This line was kindly

provided by Dr. Chapman on July 22, 2011. Biological properties of the parent strain of *E. meleagridis* USAR97-01 have been reported previously (Matsler and Chapman 2006).

Single oocyst–derived lines of *E. gallopavonis* and *E. meleagridis* were propagated in specific parasite free poults in CAF Isolation (Campus Animal Facility, University of Guelph, Guelph, ON, Canada). Turkey poults were provided feed and water ad libitum; all experimental manipulations were reviewed and approved by the University of Guelph's Animal Care Committee and complied with the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals (2nd edition). A single-oocyst line was derived from the *E. gallopavonis* Weybridge and *E. meleagridis* USAR97-01 isolates by following the method of Remmler and McGregor (1964) with the modification of using agar pieces to carry a single oocyst to be delivered to the bird orally by gelatin capsules. Sporulation of the oocysts, measurements of oocysts, sporocysts, and endogenous stages, and characterization of the endogenous life cycle stages were done using methods described previously (El-Sherry et al. 2014a, 2014b). For obtaining tissues for redescription of the endogenous stages, 12 to 14 18-day-old poults (Hybrid Turkeys, Kitchener, ON, Canada) were housed in isolation facilities with continuous lighting and provided food and water ad libitum. Poults were infected individually with increasing oocyst doses every 8 h and then killed humanely to obtain tissue samples throughout the endogenous development of this strain (see Table 1). For each bird, the intestinal tract was removed and pieces were taken from eight locations: (1) middle of the descending duodenum, (2) middle of the ascending duodenum, (3) jejunum approximately 3 cm proximal to Meckel's diverticulum, (4) jejunum approximately 3 cm distal to Meckel's diverticulum, (5) midpoint of the ileum, (6) proximal 1 cm of the cecal neck, (7) middle of the cecal pouch, and (8) middle of the rectum. Samples were placed in freshly prepared Serra fixative solution then stained with hematoxylin and eosin (Animal Health Laboratory, University of Guelph).

Table 1 Inoculating doses and timing of experimental infections with *Eimeria gallopavonis* (Weybridge strain) and *Eimeria meleagridis* (USAR97-01) in 18-day-old poults used to examine life cycle stages of the parasite

Species	Bird number	Inoculation dose (oocysts/bird)	Hours post-inoculation for tissue collection
Eimeria gallopavonis (Weybridge strain)	1, 2, 3, 4	10×10^6	32, 40, 48, 56
	5, 6, 7	5×10^5	64, 72, 80
	8, 9, 10	5×10^5	88, 96, 104
	11, 12, 13	3×10^5	112, 120, 136
	14	1×10^4	144
Eimeria meleagridis (USAR97-01)	1, 2, 3, 4	6×10^6	32, 40, 48, 56
	5, 6, 7	3×10^5	64, 72, 80
	8, 9, 10	3×10^5	88, 96, 104
	11, 12	3.5×10^4	112, 120

Results

Species redescription

Eimeria gallopavonis Hawkins 1952

Figures 1a, b, 2, 3, 4, and 5; Tables 1, 2, 4, and 6

Taxonomic summary:

Apicomplexa
Conoidasida
Coccidia
Eucoccidiorida
Eimeriorina
Eimeriidae
Eimeria gallopavonis (Hawkins 1952)

Geographic origin and date of isolation of parent culture: Central Veterinary Laboratory (CVL), Weybridge, UK, 1952

Type host: *Meleagris gallopavo* (Aves, Galliformes, Phasianidae, Meleagridinae)

Type locality: Worcester County, Massachusetts, 42.276388, -71.720917 (Hawkins 1952)

Other localities: Arkansas, USA; Guelph, Ontario, Canada—likely cosmopolitan in turkey flocks

Strain designation: Weybridge

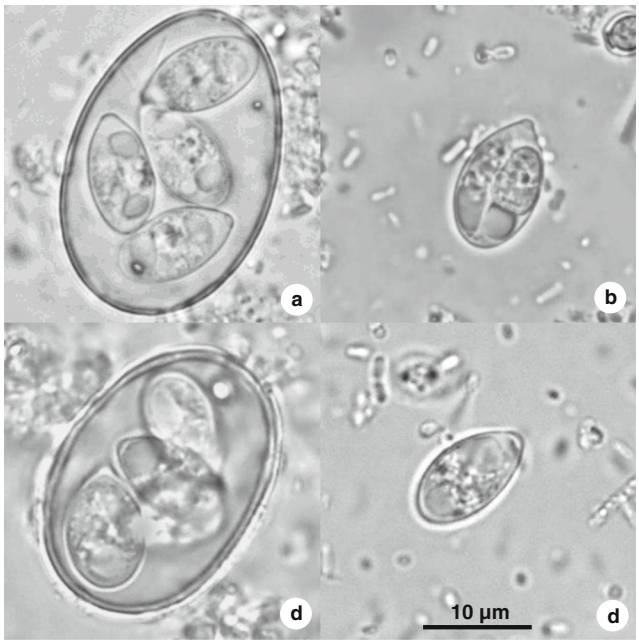

Fig. 1 Sporulated oocysts and sporocysts of *Eimeria* gallopavonis (**a**, **b**) and *Eimeria meleagridis* (**c**, **d**): *Eimeria gallopavonis*. **a** Oocyst is oval in shape with a doubled-contoured appearance to the oocyst wall and multiple polar granules can be seen at one end of the oocyst. **b** Mature sporozoites within sporocysts with fully formed Stieda bodies. **c** *Eimeria meleagridis* oocyst is ovoid with a consistent narrowing at one end and a double-layered, smooth oocyst wall. A single refractile polar granule could be detected attached to the inner layer at the narrow end. **d** Sporocysts were ellipsoidal; each sporozoite possessed both a Stieda and sub-Stieda body. Scale bar = 10 µm

Description of sporulated oocysts

Oocysts were broadly ovoid with a consistent narrowing at one end (Fig. 1a). Sporulated oocysts measured 27 ± 1.9 μm (24–31) × 19 ± 1.4 μm (15–21, $n = 30$) with a shape index of 1.4 ± 0.1. Multiple refractile polar granules (up to four granules) can be detected in sporulated oocysts. The sporulation is exogenous and takes about 48 h at 26 °C. Sporocysts measured 12 ± 0.6 μm (11–13) × 8 ± 0.5 μm (7–9, $n = 30$) (Fig. 1b). The scatter plot of lengths and widths for the measured oocysts and sporocysts all supported the presence of a morphometrically consistent population (Fig. 2).

Histological assessment of endogenous stages

The life cycle stages of *E. gallopavonis* that were observed microscopically and their locations throughout endogenous development are summarized in Table 2; the locations of asexual and sexual stages are illustrated diagrammatically in Fig. 3a, b, respectively.

First asexual generation At 32 h post-inoculation (HPI), sporozoites and trophozoites were found invading the enterocytes alongside the villi in jejunum (before and after Meckel's diverticulum), ileum, cecal neck, and rectum, with the highest number concentrated in cecal neck and rectum. The growth of the 1st generation under the cell nucleus appear to alter the host cell shape as it looked swollen, and the nucleus was pushed up toward the intestinal lumen Fig. 4a. No parasites were detected in the deep glands. The earliest mature 1st-generation meronts were detected at 48 HPI and appear to consist of ~ 200–400 merozoites. Mature 1st-generation meronts measured on average 21 μm (15–27) × 15 μm (10–21). Mature 1st-generation meronts were most numerous at 48 HPI but this stage continued to be observed until 72 HPI. No parasites were observed in the cecal pouch area.

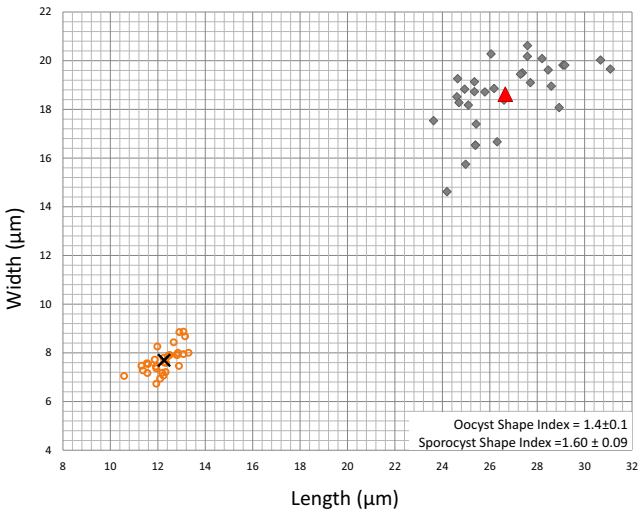

Fig. 2 Scatter plot of length and width measurements (μm) of 30 oocysts of *Eimeria gallopavonis* (closed diamonds; mean, filled triangle) and sporocysts (open circles; mean, multiplication sign)

Table 2 Location and relative abundance of *Eimeria gallopavonis* (Weybridge strain) life cycle stages in histological sections

HPI	DD	AD	JBM	JAM	MI	CN	MC	MR
32	–	–	–	+	++	++	–	++
40	–	–	–	++	++	++	–	++
48	–	–	++	++	++	++	–	++
56	–	–	+	++	+++	++	–	++
64	–	–	+	++	+++	+++	–	++
72	–	–	–	+	+++	+++	–	+++
80	–	–	+	+++	+++	+++	+	+++
88	–	–	++	+++	+++	+++	+	+++
96	–	–	++	+++	+++	+++	+	+++
104	–	–	+	+++	+++	+++	+	+++
112	–	–	++	+++	+++	+++	++	+++
120	–	–	++	+++	+++	+++	++	+++
136	–	–	–	+++	+++	+++	–	+++
144	–	–	–	+++	+++	+	–	+++

+, ++, and +++ indicate an increasing relative abundance of parasites in different regions of the intestinal tract at one time point

DD descending duodenum, *AD* ascending duodenum, *JBM* jejunum before Meckel's diverticulum, *JAM* jejunum after Meckel's diverticulum, *MI* middle of ileum, *CN* cecal neck, *MC* middle of the cecum, *MR* middle of rectum

Second asexual generation The earliest 2nd-generation trophozoites were detected at 56 HPI in jejunum (mainly below

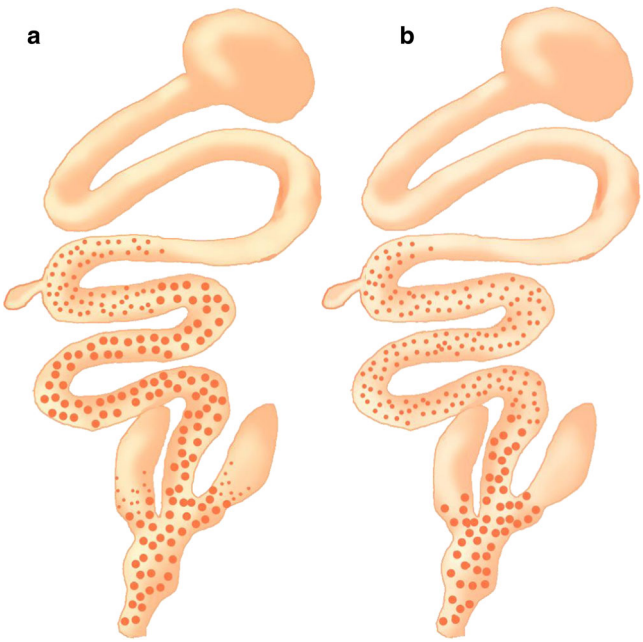

Fig. 3 Diagram illustrating the relative abundance of life cycle stages of *Eimeria gallopavonis* within an infected turkey. **a** Asexual stages—mainly starting from posterior of the yolk stalk, ileum, cecal neck (rarely cecal pouches), and rectum with the highest density of the parasites present in lower ileum, cecal neck, and rectum. **b** Sexual stages—found throughout the jejunum and ileum, more abundant in the cecal neck and rectum but not observed in the cecal pouches

Meckel's diverticulum), ileum, cecal neck, and rectum. Again, no parasites were observed in the cecal pouch area. Merogonic stages were located above the nucleus beneath the brush border of the enterocytes, alongside the villi and in the villi tips (Fig. 4b). Mature 2nd-generation meronts were first observed at 64 HPI and were most numerous at 72 HPI and measured on average 7 μm (6–12) × 5 μm (4–8). Mature meronts appeared to consist of 6–8 merozoites. Upon maturation and release of merozoites, mucosal damage became evident. Second-generation stages continued to be observed until 80 HPI.

Third asexual generation Numerous 3rd-generation trophozoites were detected at 80 HPI alongside the villi and in the tips of enterocytes in the jejunum, ileum, cecal neck, and rectum (Fig. 4c). A few scattered trophozoites were detected at this stage in the cecal pouch, and increased numbers of endogenous stages were observed in the jejunum above Meckel's diverticulum. The few parasites detected in the cecal pouch appeared to be at an earlier state of development than stages belonging to the same generation observed in other locations in the intestinal tract at the same time of collection. Numerous parasites infected the jejunum below Meckel's diverticulum, as well as within the ileum, cecal neck and rectum were more or less uniformly developed. Trophozoites of the 3rd generation were observed within the deep crypts and glands as well as alongside the villi and in the tips. Mature 3rd-generation meronts were first observed at 88 HPI and most numerous at 96 HPI. Mature meronts measured 10 μm (7–10) × 8 μm (6–11) on average. Each mature 3rd-generation meront contained about 6–16 merozoites. Maturing 3rd-generation merogonic stages were detected until 104 HPI. No evidence was observed that the few trophozoites observed in the cecal pouches ever successfully developed to mature meronts.

Gametogonic development Immature gamonts were first observed at 104 HPI. Gamonts were observed both along the sides of the villi up to the tips and in deep glands in jejunum,

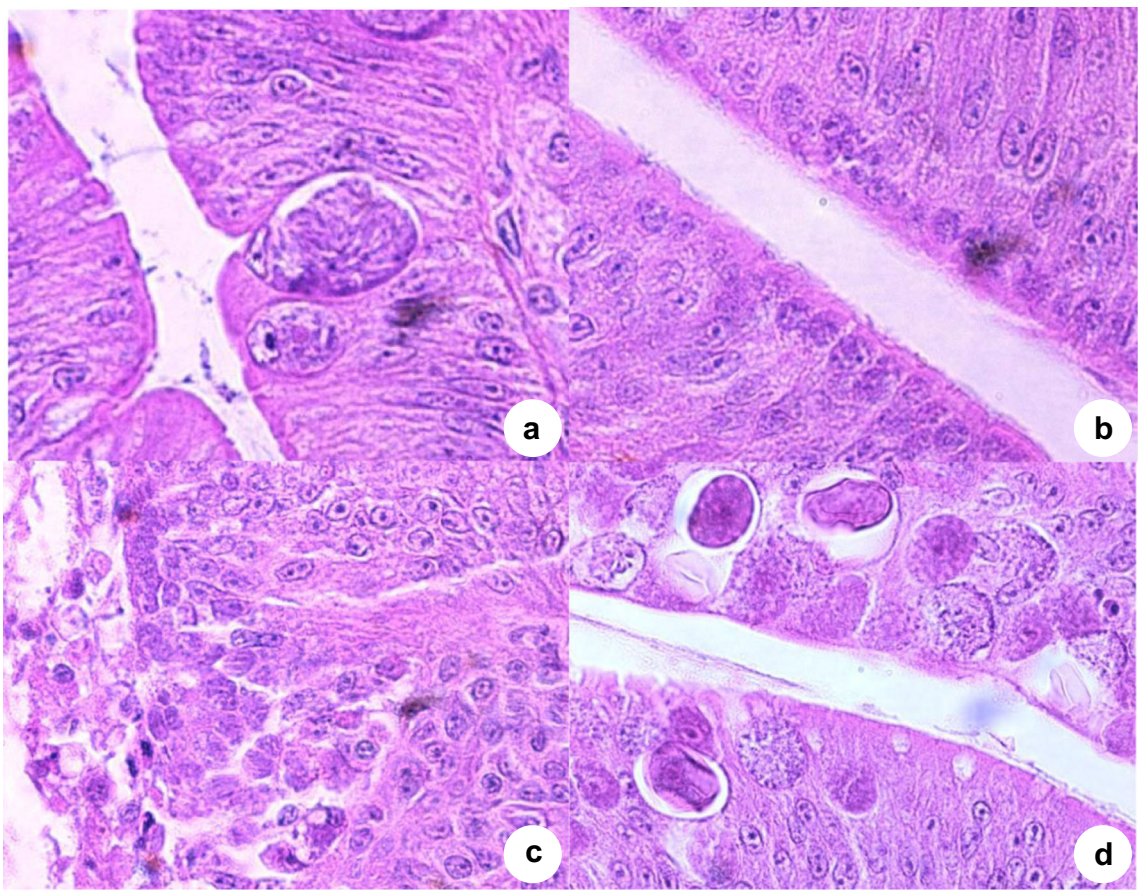

Fig. 4 Endogenous development of *Eimeria gallopavonis* in turkey poults. **a** Forty-eight HPI, large mature 1st-generation meronts located alongside the villi in jejunum (before and after Meckel's diverticulum), ileum, and cecal neck. Meronts were located below the host cell nuclei. Infected enterocytes looked swollen, and their nuclei were displaced toward the lumen. **b** Seventy-two HPI, mature 2nd-generation meronts mainly in jejunum (mainly after Meckel's diverticulum), ileum, cecal neck, and rectum. They were located above the nucleus and immediately beneath the brush border of the enterocytes alongside the villi and within the villar tips. **c** Eighty-eight HPI, numerous mature 3rd-generation meronts were observed in the crypts as well as alongside the villi and villar tips of the jejunum, ileum, cecal neck, and rectum. **d** One hundred and thirty-three HPI, unsporulated oocysts and mature gamonts were plentiful

ileum, cecal neck, and rectum (Fig. 4d). Mature gamonts and oocysts were numerous at 133 HPI. Macrogamonts measured on average 15.3 μm (13–18) × 12 μm (9–15). Mature microgamonts measured on average 17 μm (14–22) × 12 μm (10–14). Mature gamonts and unsporulated oocysts were detected until 144 HPI.

Pathological lesions

The Weybridge strain of *E. gallopavonis* is highly pathogenic. Necrosis, edema, and sloughing of the epithelium were evident and associated with characteristic white caseous material in the lumen (consisting of dead host cells as well as gamonts and oocysts) that could be observed from the serosal surface, mainly in the ileocecal junction (ileum, cecal neck, and rectum) (Fig. 5a). At a dose of 3×10^4 oocysts/bird, the caseous material could be detected filling the lumen of intestinal tract starting from Meckel's diverticulum through to the rectum (Fig. 5b). Petechial hemorrhage was rarely observed on affected mucosal surfaces. The cecal pouches usually appeared unaffected (Fig. 5b), but in rare cases, the caseous material sometimes filled the cecum. However, there was no endogenous development within the epithelial tissues lining this area suggesting that the caseous material found rarely in cecal pouches during infections with *E. gallopavonis* had been produced elsewhere and subsequently moved into the pouches.

Reference molecular data

Molecular data specifically related to *E. gallopavonis* (Weybridge strain) have been published previously (El-Sherry et al. 2015). The sequence data specifically associated with *E. gallopavonis* for which the biological redescription is provided herein are as follows:

Mitochondrial cytochrome *c* oxidase subunit I (mt COI) partial sequence KJ526132

El-Sherry et al. (2014b) amplified and sequenced a portion of the mitochondrial COI gene from this line of *E. gallopavonis* Weybridge strain.

Complete mt genome sequence KJ608413

Ogedengbe et al. (2014) provided a complete mitochondrial genome sequence obtained from direct sequencing of PCR products.

Designation of a neotype and type deposition

The neotype consists of a hapantotype series of four histological preparations of the intestinal tract of experimentally infected turkeys and a phototype of sporulated oocysts as follows: CMNPA 2018-0060.1, 48 HPI demonstrating mature 1st-generation meronts; CMNPA 2018-0060.2, 72 HPI demonstrating mature 2nd-generation meronts; CMNPA 2018-0060.3, 96 HPI demonstrating 3rd-mature generation meronts; CMNPA 2018-0060.4, 136 HPI demonstrating mature macrogamonts, microgamonts, and unsporulated oocysts; and CMNPA 2018-0060.5, a phototype of the sporulated oocyst of *Eimeria gallopavonis*.

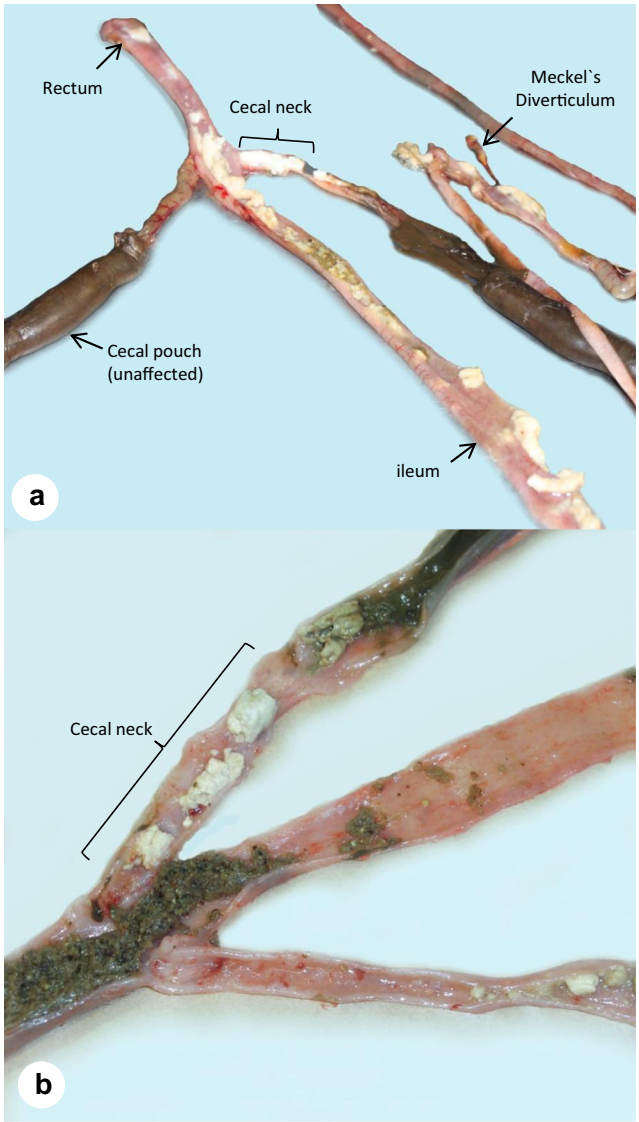

Fig. 5 Appearance of macroscopic lesions of *Eimeria gallopavonis*. **a** Lesions and caseous material were found from just above Meckel's diverticulum in the lower jejunum through to the ileum, cecal neck, and rectum. **b** Characteristic caseous material was found mainly in the cecal neck while the mucosa and the contents of the cecal pouch looked completely unaffected. Poults were inoculated with 3×10^4 oocysts/bird: illustrated lesions observed at 5 days post-inoculation

Species redescription

Eimeria meleagridis Tyzzer 1929

See Figs. 1, 6, 7, 8, and 9; and Tables 1, 3, 5, and 6

Taxonomic summary:
Apicomplexa
Conoidasida
Coccidia
Eucoccidiorida
Eimeriorina
Eimeriidae
Eimeria meleagridis (Tyzzer 1929)

Geographic origin and date of isolation of parent culture: Turkey farm in Northwest Arkansas, USA, 1997

Type host: *Meleagris gallopavo* (Aves, Galliformes, Phasianidae, Meleagridinae)

Type locality: Unknown, presumed near Cambridge, MA, USA or environs, near 42.337, -71.103 (see Tyzzer 1929)

Other localities: Cache County, Utah, USA (Hawkins 1952), UK (Clarkson 1959); Arkansas, USA (Matsler and Chapman 2006); Guelph, Ontario, Canada—likely cosmopolitan in turkey flocks

Strain designation: USAR97-01

Oocyst morphometrics

Sporulated oocysts (Fig.1c) were ovoid with a consistent, but modest, narrowing at one end. The double-layered oocyst wall was smooth. A single refractile polar granule could be detected attached to the inner layer at the narrower end of the oocyst. Sporulated oocysts measured 26.3 ± 1.5 μm (23–28) × 16.9 ± 1.2 μm (15–20, $n = 30$) with a shape index of 1.55 ± 0.11 (Fig. 6). Sporocysts were ellipsoidal, 11 ± 0.5 μm (10–12) × 6 ± 0.3 μm (6–7, $n = 30$), and each possessed both a Stieda and sub-Stieda body (Fig.1d).

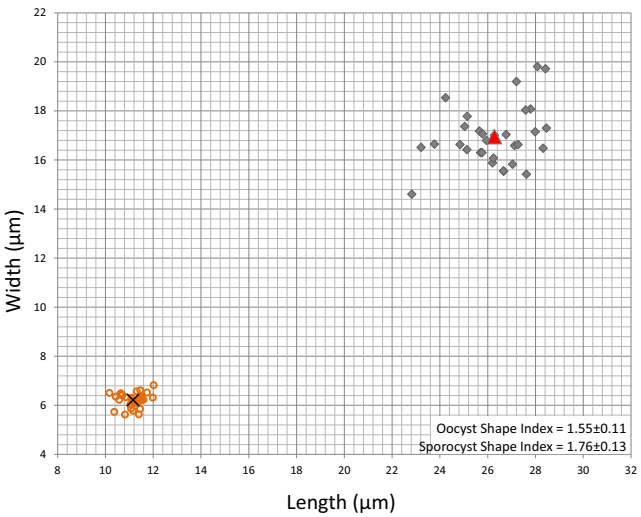

Fig. 6 Scatter plot of length and width measurements (μm) of 30 oocysts of *Eimeria meleagridis* (closed diamonds; mean, filled triangle) and sporocysts (open circles; mean, multiplication sign)

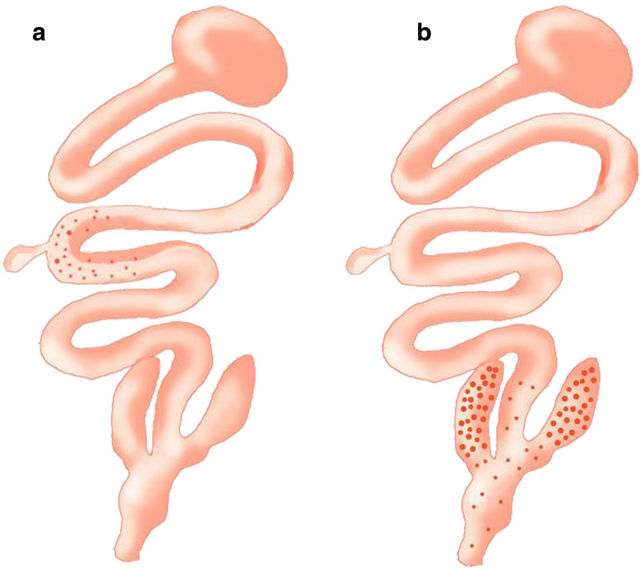

Fig.7 Diagram illustrating the relative abundance of life cycle stages of *Eimeria meleagridis* within an infected turkey. **a** First asexual generation was observed mainly in the jejunum around the yolk stalk. **b** Second, 3rd asexual generations, and sexual stages were confined mainly to the cecal pouches with few parasites observed in the ileum, cecal necks, or rectum

Histological assessment of endogenous stages

The endogenous life cycle stages of *E. meleagridis* that were observed microscopically and their locations throughout development are summarized in Table 3, and the locations of asexual and sexual stages are illustrated diagrammatically in Fig. 7a, b, respectively.

Table 3 Location and relative abundance of *Eimeria meleagridis* (USAR97-01) life cycle stages in histological sections

HPI	DD	AD	JBM	JAM	MI	CN	MC	MR
32	–	–	–	+	–	–	–	–
40	–	–	–	–	–	–	–	–
48	–	–	+	+	+	–	–	–
56	–	–	+	+	+	–	+	–
64	–	–	+	+	+	+	++	+
72	–	–	–	–	–	+	+++	–
80	–	–	–	–	–	–	+++	–
88	–	–	–	–	–	–	+++	–
96	–	–	–	–	++	++	+++	++
104	–	–	–	–	+	+	+++	++
112	–	–	–	–	–	+	+++	–
120	–	–	–	–	–	+	+++	+++

+, ++, and +++ indicate an increasing relative abundance of parasites in different regions of the intestinal tract at one time point

DD descending duodenum, *AD* ascending duodenum, *JBM* jejunum before Meckel's diverticulum, *JAM* jejunum after Meckel's diverticulum, *MI* middle of ileum, *CN* cecal neck, *MC* middle of the cecum, *MR* middle of rectum

First asexual generation At 32 HPI, sporozoites were found invading the enterocytes alongside the villi and near the tips in jejunum (before and after Meckel's diverticulum) and ileum. The growth of the 1st generation above or below the cell nucleus led to swelling of the host cell, and the nucleus appeared more flat and pushed above or below the growing schizonts (Fig. 8a). No parasites were detected in the deep glands. The earliest mature 1st-generation meronts were detected at 48 HPI and appeared to consist of 50–70 merozoites. Mature 1st-generation meronts measured on average 14 μm (12–18) × 12 μm (9–15). Mature 1st-generation meronts were most numerous at 48 HPI, but this stage continued to be observed until 72 HPI. Neither sporozoites nor 1st-generation merogonic stages were observed within the cecal neck or cecal pouch areas.

Second asexual generation The earliest 2nd-generation trophozoites were detected at 56 HPI mainly in the cecal pouch. A few parasites were observed rarely in the cecal neck or rectum. They were located above the nucleus beneath the brush border of the enterocytes. No 2nd-generation stages were detected in the deep glands (Fig. 8b). Most 2nd-generation meronts matured at about 64 HPI and measured on average 9 μm (6–12) × 7 μm (5–10) and contained 6–8 merozoites. Mature 2nd-generation meronts were most numerous at 64 HPI, but few mature 2nd-generation meronts were observed until 80 HPI.

Third asexual generation Huge number of trophozoites of 3rd generation was detected at 80 HPI under the brush border of villar enterocytes (not deeply in the crypts) in the cecal pouch exclusively (Fig. 8c). No parasites were detected in the cecal necks or rectum. Upon release of the meronts from this stage, severe mucosal damage was observed in the cecal pouch area. Mature 3rd-generation meronts were observed at 88 HPI and measured on average 8 μm (7–12) × 7 μm (6–9), and each contained about 6–12 merozoites. Mature 3rd-generation meronts were most numerous at 88 HPI, but this stage continued to be observed until 104 HPI.

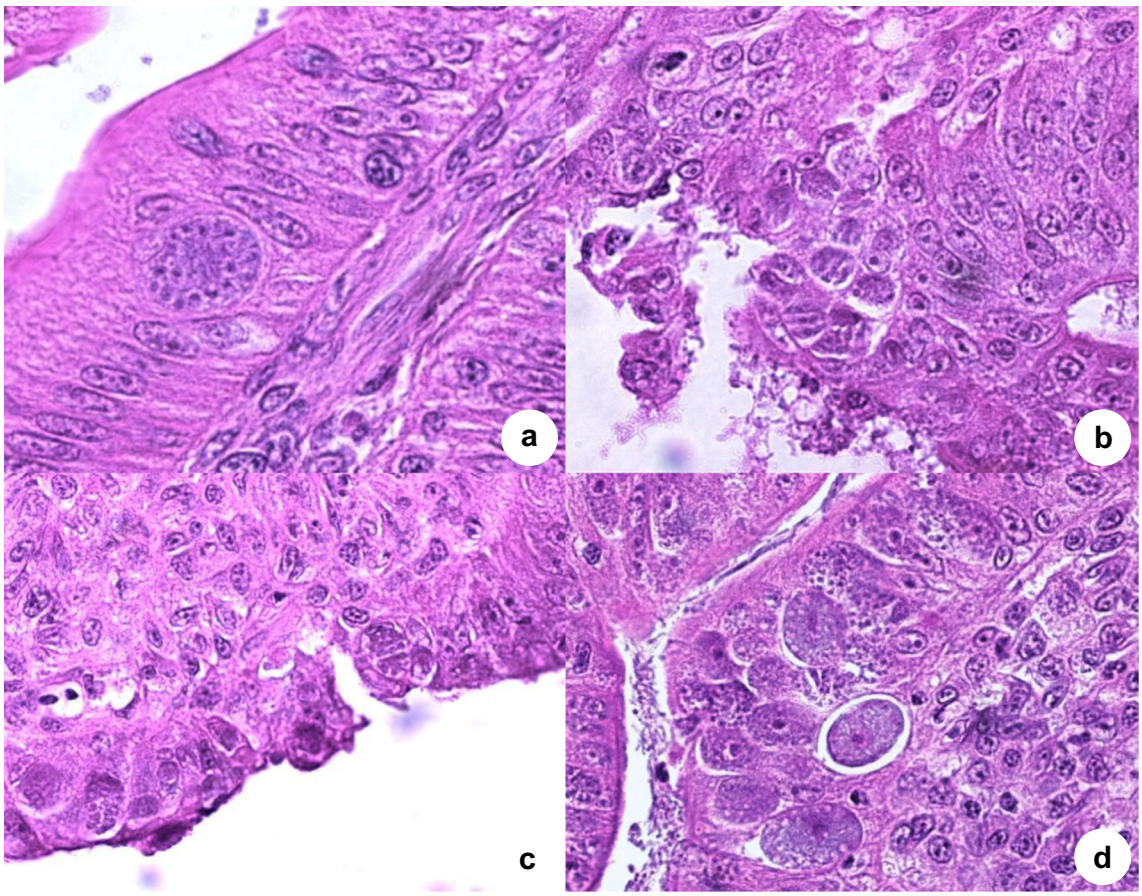

Fig. 8 Endogenous development of *Eimeria meleagridis* in turkey poults. **a** Forty-eight HPI, mature 1st-generation meronts invaded enterocytes along the sides of the villi in the jejunum. The growth of the 1st-generation meronts above or below the cell nucleus led to swelling of the host cell, and its nucleus appeared flattened and displaced above the growing meronts. **b** Sixty-four HPI, mature 2nd-generation meronts in the cecal pouch were located above the nucleus beneath the brush border of the enterocytes. **c** Eighty HPI, 3rd-generation meronts were detected under the brush border of villar enterocytes (not in the deep glands), mainly in the cecal pouch. **d** One hundred and twelve HPI, numerous mature female and male gamonts were observed mainly in enterocytes lining the villar tips, but a few parasites were found in deep glands

Gametogonic development Immature gamonts were first observed at 96 HPI in the ileum, cecal neck, cecal pouch, and rectum. Gamonts were observed mainly in villar enterocytes, but a few parasites were found within the crypt epithelium (Figs. 8d). Mature gamonts were observed first at 112 HPI. Large numbers of mature gamonts and a few unsporulated oocysts were observed at 120 HPI. Mature female gamonts measured on average 15 μm (12–19) × 10 μm (8–13). Mature male gamonts measured on average 15 μm (12–21) × 11 μm (8–14).

Pathological lesions

The pathological lesions associated with this strain of *E. meleagridis* were confined to the cecum. At a dose of 2×10^4 oocysts/bird, the two ceca looked swollen and edematous with a marked corrugation of the serosal surface. Opening the cecal pouch revealed creamy colored caseous material that varied in consistency from caseous loose material to a granular caseous core filling the whole lumen (Fig. 9a). The mucosa was thickened markedly, and there were rows of petechial hemorrhages in the cecal pouch area; in contrast, the epithelium lining the cecal neck was not involved (Fig.9b).

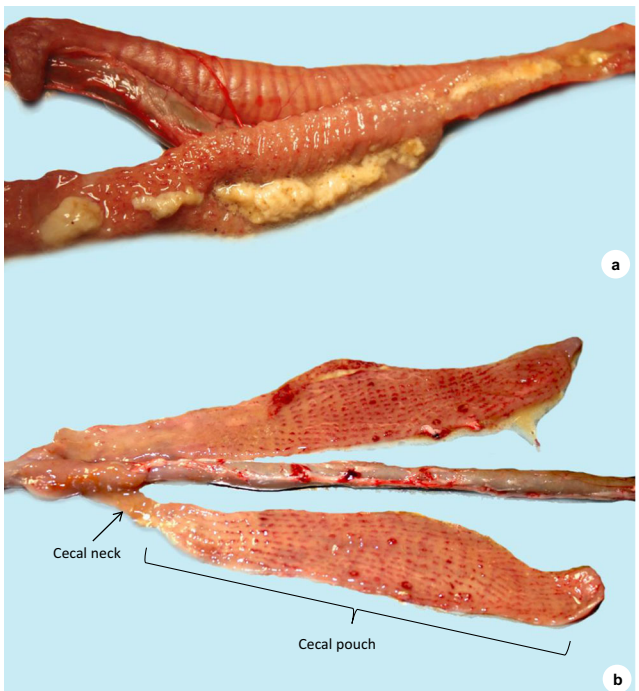

Fig. 9 Appearance of macroscopic lesions of *Eimeria meleagridis*. **a** Corrugated appearance of the serosal surface of the affected cecal pouch (background) and caseous material filling the opened cecal pouch (foreground). **b** The exposed mucosa usually appeared edematous and inflamed with characteristic petechial hemorrhages found in rows in the cecal pouches. Lesions did not extend into the cecal necks even in heavily infected ceca. Poults were inoculated with 2×10^4 oocysts/bird: illustrated lesions observed at 5 days post-inoculation

Reference molecular data

Molecular data specifically related to *E. meleagridis* (USAR97-01) have been published previously (El-Sherry et al. 2015). The sequence data specifically associated with *E. meleagridis* for which the biological redescription is provided herein are as follows:

Mitochondrial cytochrome *c* oxidase subunit I (mt COI) partial sequence. KJ526133

El-Sherry et al. (2015) amplified and sequenced a portion of the mitochondrial COI gene from *E. meleagridis* (USAR97-01).

Complete mt genome sequence KJ608418

Ogedengbe et al. (2014) provided a complete mitochondrial genome sequence obtained from direct sequencing of PCR products.

Designation of a neotype and type deposition

The neotype for *Eimeria meleagridis* consists of a hapantotype series of four histological preparations of the intestinal tract of experimentally infected turkeys and a phototype of sporulated oocysts as follows: CMNPA 2018-0061.1, 56 HPI demonstrating mature 1st-generation meronts; CMNPA 2018-0061.2, 64 HPI demonstrating mature 2nd-generation meronts; CMNPA 2018-0061.3, 88 HPI demonstrating 3rd-mature generation meronts; CMNPA 2018-0061.4, 120 HPI demonstrating mature macrogamonts, microgamonts, and unsporulated oocysts; and CMNPA 2018-0061.5, a phototype of the sporulated oocyst of this parasite.

Discussion

The three *Eimeria* species that are responsible for cecal coccidiosis in turkeys are *E. adenoeides*, *E. gallopavonis*, and *E. meleagridis*. Biological redescriptions of these three *Eimeria* species (El-Sherry et al. 2014a, 2014b; present study), including genotyping of mitochondrial COI and nuclear 18S rDNA loci (see Ogedengbe et al. 2014; El-Sherry et al. 2015), were undertaken to provide detailed information for species differentiation. These three parasite species can be differentiated using the combination of (1) oocyst dimensions and characteristics, (2) endogenous development, (3) gross pathological lesions, and (4) genotyping.

Oocyst dimensions The Weybridge strain of *E. gallopavonis* was obtained as an isolate named "*E. adenoeides* (Weybridge strain)"; this strain was isolated originally at the Central Veterinary Laboratory, Weybridge, UK in 1952 (Chapman, pers. comm.; Hein 1969). Our first observation regarding this strain was that its oocysts were larger than those of

E. adenoeides Guelph strain that was isolated and characterized earlier (El-Sherry et al. 2014b). Oocysts of the Weybridge strain of *E. adenoeides* were elongate and ellipsoidal in shape, with a doubled-contoured oocyst wall, that measured 27 ± 1.9 μm (24–31) × 19 ± 1.4 μm (16–21) with a shape index of 1.4 ± 0.1. The morphometrics of the oocysts more closely matched *E. gallopavonis* Hawkins 1952 but could be confused with *E. meleagridis* Tyzzer 1929 as well because oocysts of the latter species are morphometrically similar to *E. gallopavonis* (see Matsler and Chapman 2006). In previous work (El-Sherry et al. 2015), single oocyst–derived lines for each of these species were isolated and purified; subsequent characterization of these new lines, both molecularly (using mt COI sequences; see El-Sherry et al. 2015) and morphometrically, permit the unambiguous differentiation among these *Eimeria* species. These studies concluded that the oocysts of *E. adenoeides* are smaller than those of *E. gallopavonis* or *E. meleagridis*. Although the dimensions of oocysts of *E. meleagridis* and *E. gallopavonis* overlap, the oocysts of the latter two species could be differentiated by the number of polar granules present in the sporulated oocyst (see Fig. 1a, c; El-Sherry et al. 2015).

Endogenous development Hawkins (1952) formally described *E. gallopavonis* but provided only a brief description regarding endogenous stages and provided no information about endogenous development prior to 3 days post-inoculation (DPI) (Table 4). According to Hawkins (1952), the 1st generation was detected at 72 HPI and 1st-generation meronts contained eight merozoites. Farr (1964), in a more detailed study on the same species, reported the mature 1st-generation meronts at 72 HPI to be large (35–40 μm × 28–30 μm) and to contain at least 400 merozoites. The growth of the 1st-generation meronts below the host cell nuclei altered the host cell shape and pushed the host cell nucleus toward the intestinal lumen. Our observations were similar in the present redescription of *E. gallopavonis* except that mature 1st-generation stages were detected as early as 48 HPI. These meronts measured on average 21 μm (15–27) × 15 μm (10–21) and contained ~ 200–400 merozoites. Hawkins (1952) detected mature 2nd-generation meronts at 96 HPI that appeared to be of two different types, large and small, but he did not provide any information about the dimensions of these forms. Farr (1964) recorded mature 2nd-generation meronts measuring 9 × 9 μm containing 8–12 merozoites at 96 HPI in both villar and crypt epithelium. Doran and Augustine (1977) found it difficult to assign stages to a particular generation of development after 48 HPI, because all meronts of *E. gallopavonis* stained similarly and the meronts and merozoites of different "generations" were of about the same dimensions. In the present study, 2nd-generation trophozoites were detected as early as 56 HPI and mature 2nd-generation meronts measuring 7 × 5 μm and containing ~ 6–8 merozoites were numerous at 72 HPI. Hawkins (1952), Farr (1964), and Doran and Augustine (1977) did not describe a 3rd-asexual generation for *E. gallopavonis*. Doran and Augustine (1977), however, did not exclude the probability of the presence of a 3rd generation of merogonic replication; they recorded merogonic replication until 144 HPI suggesting that there were more than two asexual generations. In the present work, trophozoites of the 3rd generation were observed at 80 HPI and mature 3rd-generation meronts were numerous at 88 HPI. Differentiation between 2nd and 3rd generations was facilitated by the observation that the 3rd-generation stages invaded both crypt and villar enterocytes, unlike the 2nd-asexual generation. It is likely that the meronts described by Farr (1964) at 96 HPI in deep glandular epithelium, which he called 2nd-generation meronts, were actually the 3rd-generation stages detected in the present study; it is probable that the earlier 2nd-generation was not observed by Farr. A few trophozoites were found in the enterocytes of the cecal pouch at 80 HPI. These few trophozoites were developmentally delayed compared with other 3rd-generation stages that developed in ileum, cecal neck, and rectum. Examination of sections from subsequent time points did not reveal any further development for such trophozoites, and they ultimately disappeared. It is possible that a paucity of suitable enterocytes in more typically parasitized regions of the intestinal tract forced a few parasites to infect a region of the digestive tract to which they were not suited. The earliest unsporulated oocysts were detected in tissues at about 133 HPI, and this was close to the prepatent period described for *E. gallopavonis* in the original description (144 h by Hawkins 1952) and confirmed later by Farr (1964).

Eimeria meleagridis Tyzzer 1929 was the first *Eimeria* species described to infect the lower intestinal tract in turkeys. In his brief species description, Tyzzer (1929) observed that the lesions induced by this species were mainly confined to the cecal pouch; only limited observations on the superficial development of this parasite in surface epithelium, rather than deep glandular epithelium and the shedding of oocysts 5 DPI was reported. Hawkins (1952) was the first to describe the life cycle of *E. meleagridis* in any detail. Hawkins described the presence of 1st-generation meronts in the cecum that measured ~ 5 μm in diameter and contained about six to eight nuclei. Hawkins (1952) also detected 2nd-generation meronts containing about 10–14 merozoites in the ceca (probably referring to the cecal pouches) at about 73 HPI and concluded that the 3rd generation of endogenous development was gametogony at about 4–5 DPI (gamonts measured 10–20 μm). Clarkson (1959) conducted a more detailed study on a strain of *E. meleagridis* isolated in England and was the first to report the presence of the large (~ 15–20 μm) 1st-generation meronts in the enterocytes of the jejunum around the yolk stalk. Clarkson (1959) detected mature 1st-generation meronts at 48 HPI and observed about 50–100 merozoites in each. The 2nd-generation (merogonic) and 3rd-generation

Table 4 Comparison of endogenous development of *Eimeria gallopavonis* described by various authors

Features	Studies on endogenous development of *Eimeria gallopavonis* Hawkins 1952			Present study
	Hawkins (1952)	Farr (1964)	Vrba and Pakandl (2014)	
Oocyst size (L × W)	27.1 μm (22.2–32.7) × 17.23 μm (15.2–19.4)	–	26.6 ± 2.2 μm × 16.4 ± 1.1 μm	27 μm (24–31) × 19 μm (15–21)
Prepatent period	144 HPI (in feces)	144 HPI (in feces)	126–132 HPI	133 HPI (unsporulated oocysts in tissue)
Mature 1st generation				
Size	–	(35–40) × (28–30) μm	–	21 μm (15–27) × 15 μm (10–21)
Intestinal location	Ileum, cecum, and rectum	Posterior ileum, tubular ceca, and rectum	Ileum, caecum, and rectum	Jejunum (above and below Meckel's), ileum, cecal neck, and rectum
Histological location	–	Under the host cell nucleus and push the nucleus toward lumen	Villi	Beneath cell nucleus along sides of villi, not in deep glands
Number of merozoites	~ 8	~ 400	~ 60–100	~ 200–400
Time when first observed	72 HPI	72 HPI	48 HPI	48 HPI
Mature 2nd generation				
Size	Large–small	9 × 9 μm	–	7 μm (6–12) × 5 μm (4–8)
Intestinal location	Ileum, cecum, and rectum	Posterior ileum, tubular ceca, and rectum	Ileum, cecum, and rectum	Jejunum (mainly after Meckel's), ileum, cecal neck, and rectum
Histological location	–	Above and below the host cell nuclei, alongside the villi, and in villar tips as well as in glandular epithelium	Villi	Located above nucleus beneath brush border of enterocytes alongside the villi and within villar tips
Number of merozoites	–	~ 8–12	~ 8–12	~ 6–8
Time when first observed	96 HPI	96 HPI	64–96 HPI	64 HPI
Mature 3rd generation				
Size	–	–	–	10 μm (7–10) × 8 μm (6–11)
Intestinal location	–	–	Ileum, cecum, and rectum	Jejunum, ileum, cecal neck, and rectum
Histological location	–	–	Villi	Invading the deep crypts and glands as well as alongside the villi and in villar tips
Number of merozoites	–	–	~ 10–18	~ 6–16
Time when first observed	–	–	96–112 HPI	88 HPI
Mature gamonts				
Size	–	–	–	Macrogamonts 15 × 12 μm; microgamonts 17 × 12 μm
Intestinal location	–	–	Cecum and rectum	Cecal neck, cecum, and rectum
Histological location	–	In the villi, villar tips, and deep glands	Villi	Alongside villi up to tips as well as deeply within crypts
Time when first observed		114 HPI	144 HPI	112 HPI

(gametogonic) stages were observed to develop in the cecum, rectum, and ileum. Clarkson (1959) was also the first to report the presence of a small number of mature gamonts in deep glands, as well as the superficial epithelium. Sequential infection of various regions of the intestinal tract during endogenous development is not unique; development that starts in the small intestine and then moves to other regions of the intestinal tract has been described (e.g., *Eimeria necatrix* in chickens by Tyzzer et al. 1932 and *Eimeria bovis* in ox by Hammond et al. 1946). Matsler and Chapman (2006) isolated a line of *E. meleagridis* in 1997 from a turkey farm in Northwest Arkansas, USA. They identified the line as *E. meleagridis* based on the presence of large 1st-generation schizonts in the mid-intestine and the small meronts in the ceca. Repurification from Matsler and Chapman's (2006) parent strain of *E. meleagridis* and subsequent biological redescription was undertaken in the present work so that comparable studies on endogenous development of all three *Eimeria* species infecting the ceca of turkeys could be completed under the same experimental conditions. In the present study, and in the redescription of *E. adenoeides* Guelph strain (El-Sherry et al. 2014b) based on experiments conducted concurrently with the present studies, the age, breed, housing, and feed of all poults used to examine endogenous development were essentially identical; similarly, the method and sites of necropsy, processing, fixation, sectioning, and staining were the same for all three *Eimeria* species. Endogenous development observed in present study was similar to what was described by Matsler and Chapman (2006) except that only 1st-generation merogonic stages were found in the mid-intestine in the present study, and no other developmental stages were detected in this area. In the present work, three asexual generations were observed before the start of gametogony. The 2nd- and 3rd-asexual generations were found to develop exclusively in the cecal pouch. A few gametogonic stages were detected in the ileum, cecal necks, and rectum, but the majority of gametogenesis occurred within the cecal pouches, distal to the cecal necks. Gamonts developed principally within villar enterocytes, but a few were observed to infect crypt enterocytes. Our observations agree with Clarkson's (1959) interpretation of endogenous development except that Clarkson did not detect the 3rd-asexual generation. A summary of observations on *E. meleagridis* of previous workers and the present study is provided in Table 5.

Moore and Brown (1951) noted specifically that *E. adenoeides* can be differentiated from other cecal *Eimeria* species infecting turkeys by its typical invasion of crypt enterocytes during endogenous development. For *E. adenoeides* (Guelph strain), the presence of the endogenous stages in deep glands was observed starting in the 2nd-asexual generation and continuing through all subsequent endogenous stages (El-Sherry et al. 2014b). With *E. meleagridis* in the present study, only a few gametogonic stages were observed in the crypt epithelium in agreement with Clarkson's (1959) observations; Matsler and Chapman (2006) never observed stages within crypt enterocytes. Clarkson (1959), in a detailed study using a UK isolate different from both the one used by Tyzzer (1929) to name the species and the present US strain, observed endogenous development and timing that were remarkably similar to the present observations on *E. meleagridis* USAR97-01. The only exception was that the 3rd-asexual generation was not recognized by Clarkson. The development of the 2nd- and 3rd-asexual generations of *E. meleagridis* USAR97-01 overlaps somewhat, and it is likely that the maturing meronts observed by Clarkson (1959) at 70 HPI were 2nd-generation meronts, and those observed at 84 HPI were 3rd-generation meronts.

Gross pathological lesions The pathogenicity and pathological lesions induced by these three *Eimeria* species have been examined in many previous studies. Hawkins (1952) was unable to provide much information regarding the pathogenicity of *E. gallopavonis* because of the limited material available for the species description. Later studies by Farr et al. (1961) and Wehr et al. (1962) demonstrated that lesions with this parasite were usually confined to the lower part of the intestine, posterior to the yolk stalk. Lesions were located consistently around the ileocecal junction but could extend up the intestinal tract as far as the yolk stalk and down into the rectum in heavily infected birds. In the present study, the infected mucosa appeared inflamed and edematous with a creamy white exudate observed in all of the affected areas; in most cases, the caseous material was comparatively soft unlike the firm solid cecal cores observed with *E. adenoeides* (see El-Sherry et al. 2014a). The Weybridge strain of *E. gallopavonis* used in the present study is highly pathogenic. A dose of 3×10^4 oocysts was enough to induce the typical characteristic pathological lesions of *E. gallopavonis*. The lesions and the caseous material were detected along the intestinal tract starting posterior to the yolk stalk and continuing through the ileum, cecal necks, and rectum, but the cecal pouches were not involved. The Weybridge strain was used in some previous studies under the name of *E. adenoeides* (Weybridge strain) (e.g., Hein 1969; Joyner and Norton 1972). Hein's (1969) description of the lesions induced by this misnamed strain matched the lesions described previously (Farr et al. 1961; Wehr et al. 1962) for *E. gallopavonis* as well as those observed in the current study (see Fig. 5). Hein (1969) also observed that *E. gallopavonis* Weybridge can be highly pathogenic: a dose of 2×10^4 oocysts/poult was found to be sufficient to induce 70% mortality. The recognition that *E. adenoeides* (Weybridge stain) is actually a strain of *E. gallopavonis* suggests that this parasite may be more of an issue in Europe than previously assumed (Trees 2002) and that many reports of "large" strains of *E. adenoeides* in Europe may actually represent *E. gallopavonis* infections.

Table 5 Comparison of endogenous development of *Eimeria meleagridis* described by various authors

Features	Previous studies on endogenous development of *Eimeria meleagridis* Tyzzer 1929					Present study
	Tyzzer (1929)	Hawkins (1952)	Clarkson (1959)	Matsler and Chapman (2006)	Vrba and Pakandl (2014)	
Oocyst size (L × W)	23.79 × 17.38 μm	24.4 μm (20.3–30.8) × 18.12 μm (15.4–20.6)	22.5 ± 2.3 μm × 16.25 ± 1.23 μm	24.9 μm (21–31) × 17.0 μm (14–21)	22.8 ± 2.0 μm × 14.9 ± 0.9 μm	26.3 ± 1.5 μm (23–28) × 16.9 ± 1.2 μm (15–20)
Prepatent period	120 HPI	120 HPI	110 HPI	120 HPI	102–108 HPI	120 HPI
Mature 1st generation						
Size	All life cycle stages	5 × 5 μm	20 × 15 μm	20 × 15 μm	–	14 (12–18) × 12 (9–15) μm
Intestinal location	Lower intestine and ceca	Cecum	Mid-jejunum around Meckel's diverticulum	Mid-jejunum, upper, and mid-ileum	Ileum	Jejunum around Meckel's diverticulum
Histological location	surface epithelium	Superficial epithelium	Superficial epithelium	Not in deep glands	Villi and crypts	Superficial epithelium, not in deep glands
Number of merozoites		~6–8	~50–100	~60	~32–60	~50–70
Time when first observed		17 HPI	48 HPI	48 HPI	48 HPI	48 HPI
Mature 2nd generation						
Size	–	3 × 2 μm	9 × 9 μm	10.5 × 8.8 μm	–	9 (6–12) × 7 (5–10) μm
Intestinal location		Cecum	Cecum	Jejunum, upper, and middle ileum.	Cecum	Cecal pouch
Histological location		Superficial epithelium	Superficial epithelium, few in deep glands	Superficial epithelium	Villi and crypts	Under brush border above nucleus, not in deep glands
Number of merozoites		~10–14	~8–16	–	~6–10	~6–8
Time when first observed		73 HPI	60 HPI	60 HPI	64–80 HPI	64 HPI
Mature 3rd generation						
Size	–	Few go through 3rd asexual stage	–	Different meront sizes at different times: 10.5 × 7.3 μm at 60 HPI, 8.2 × 5.8 μm at 72 HPI, 6.9 × 5.8 μm at 84 HPI, 7.8 × 6.4 μm at 108 HPI; all in mid-cecum; researchers concluded at least 4 asexual generations	–	8 (7–12) × 7 (6–9) μm
Intestinal location					Cecum	Cecal pouch
Histological location					Villi and crypts	As 2nd generation
Number of merozoites					~5–11	~6–12
Time when first observed					80–96 HPI	88 HPI
Mature gamonts						
Size		20 × 10 μm	18 × 13 μm	–	–	Macrogamont 15 (12–19) × 10 (8–13) μm, microgamont 15 (12–21) × 11 (8–14) μm
Intestinal location		Cecum	Ileum, cecum, and rectum,	Ileum, cecum, and rectum	Cecum	Ileum, cecal neck, cecal pouch, and rectum
Histological location			Superficial epithelium and few in deep glands	Not in deep glands	Villi and crypts	Few parasites were detected in deep glands
Time when first observed		96–108 HPI	96 HPI	108 HPI	96 HPI	112 HPI

Table 6 Comparisons among three redescribed *Eimeria* species infecting the lower part of the intestine *Eimeria gallopavonis* Weybridge strain, *Eimeria adenoeides* Guelph strain, and *Eimeria meleagridis* USAR97-01 strain

Features	*Eimeria adenoeides* Guelph strain (El-Sherry et al. 2014b)	*Eimeria gallopavonis* Weybridge strain (present study)	*Eimeria meleagridis* USAR97-01 (present study)
Oocyst size (L×W)	19 μm (17–22) × 14 μm (13–16)	27 ± 1.9 μm (24–31) × 19 ± 1.4 μm (16–21)	26.3 ± 1.5 μm (23–28) × 16.9 ± 1.2 μm (15–20)
Prepatent period	112 h	144 h	120 h
Mature 1st generation			
Size	13 × 10 μm	21 × 15 μm	14 × 12 μm
Intestinal location	Mainly in ileum and cecal neck, fewer in rectum	Jejunum (before and after Meckel's diverticulum), ileum, cecal neck, and rectum	Jejunum around Meckel's diverticulum
Histological location	Below the host cell nuclei along the sides of the villi	Beneath cell nucleus alongside villi, not in deep glands	Superficial epithelium, not in deep glands
Number of merozoites	~ 45–50	~ 200–400	~ 50–70
Time when first observed	40 HPI	48 HPI	48 HPI
Mature 2nd generation			
Size	9 × 7 μm	7 × 5 μm	9 × 7 μm
Intestinal location	Mainly in mid-cecum, few in the ileum or cecal neck	Jejunum (mainly after Meckel's diverticulum), ileum, cecal neck, and rectum.	Cecal pouch
Histological location	Near the tips of cells above host cell nuclei, along the sides of the villi and in the crypts	Located above the nucleus beneath the brush border of the enterocytes alongside the villi and in the villi tips.	Under brush border above nucleus. Not in deep glands
Number of merozoites	~ 12–16	~ 6–8	~ 6–8
Time when first observed	56 HPI	64 HPI	64 HPI
Mature 3rd generation			
Size	10 × 10 μm	10 × 8 μm	8 × 7 μm
Intestinal location	Lower ileum, ceca, and rectum	Jejunum, ileum, cecal neck, and rectum	Cecal pouch
Histological location	Above host cell nuclei, along the sides of villi and in deep glands	Invading deep crypts and glands as well as along the sides of villi and in villar tips	As 2nd generation
Number of merozoites	~ 12–24	~ 6–16	~ 6–12
Time when first observed	80 HPI	88 HPI	88 HPI
Mature gamonts			
Size	Male and female 20 × 18 μm	Macrogamont 15 × 12 μm, microgamont 17 × 12 μm	Macrogamont 15 × 10 μm, microgamont 15 × 11 μm
Intestinal location	Posterior third of small intestine, ceca, and rectum	Cecal neck, cecum, and rectum	Ileum, cecal neck, cecal pouch, and rectum
Histological location	In the villi, villar tips, and deep glands	Alongside the villi up to the tips and deeply within the crypts	Few parasites in deep glands
Time when first observed	104 HPI	133 HPI	112 HPI

Eimeria meleagridis had been reported in previous studies to be essentially non-pathogenic (Hawkins 1952; Clarkson 1959). However, in heavily infected turkeys, macroscopic lesions caused by *E. meleagridis* could appear similar to lesions induced by *E. adenoeides* as reported by El-Sherry et al. (2014b). Clarkson (1959) could not induce any mortality with an inoculating dose as high as 1×10^6 oocysts/poult, although such heavily challenged birds developed typical caseous cores within the cecal pouches. Matsler and Chapman (2006) measured the effect of *E. meleagridis* USAR97-01 on body weight gain. They concluded that this strain has significant effects on body weights and feed conversion, although they did not observe any macroscopic lesions in the intestinal tract of the infected birds. *E. meleagridis* USAR97-01 produced the typical cecal lesions in the present study with a dose of 2×10^4 oocysts/bird that matched descriptions of lesions by this species in earlier studies by Hawkins (1952) and Clarkson (1959). The lesions observed in the current study were similar to those induced by *E. adenoeides* (Guelph strain); however, the caseous material that filled the cecal pouches during infections with *E. meleagridis* never attained the consolidated, firm consistency of the cecal cores of the former species. Of note, marked regions of petechial hemorrhage on the mucosa surface of the cecal pouch (but not the cecal neck) that were frequently arranged in rows were observed in birds infected with *E. meleagridis* in the present study. Petechial hemorrhages in the cecal mucosa of infected birds had been reported previously for *E. meleagridis* by Hawkins (1952). Macroscopic lesions produced by *E. meleagridis* seem to be confined to the cecal pouch; caseous material in other regions of the intestinal tract likely originated within the cecal pouches.

The pathological lesions induced by *E. adenoeides* Guelph strain agreed with those described originally by Moore and Brown (1951) in their description of this species. El-Sherry et al. (2014b) reported that the serosal surface of infected ceca had a corrugated appearance and contained a characteristic solid caseous plug in each cecal pouch. Caseous material was also observed by these authors in lesser amounts in the ileum, cecal necks, or rectum during moderate or severe infections. The exposed mucosa appeared slightly inflamed but only a few scattered petechial hemorrhages were ever observed, even in severely affected ceca (see Fig. 6 of El-Sherry et al. 2014b) unlike *E. meleagridis* that frequently caused numerous petechial hemorrhages on the mucosa of infected cecal pouches (Fig. 9).

Summary The distinguishing biological features for each of the three newly characterized *Eimeria* sp. lines (El-Sherry et al. 2015; present study) are summarized in Table 6. The oocysts of *E. adenoeides* Guelph strain are smaller than either *E. gallopavonis* or *E. meleagridis*. Although similarities existed among the three *Eimeria* species, especially between *E. adenoeides* and *E. meleagridis*, the endogenous development of each species was unique. All endogenous development of *E. adenoeides* Guelph strain was confined to the lower part of the intestine (ileum, cecum, and rectum). The 1st generation was detected mainly in the lower ileum and cecal necks (small meronts with 45–50 merozoites) followed by 2nd and subsequent asexual generations in the cecal necks and pouches, and concluded with gametogonic development that was found throughout the lower ileum, ceca (including cecal necks), and rectum. In contrast, the *E. meleagridis* life cycle started much higher in the digestive tract around the mid-jejunum (around Meckel's diverticulum) with medium-sized 1st-generation meronts that matured to contain 50–100 merozoites, followed by two asexual generations that developed exclusively in the cecal pouches (but not in the cecal necks) and concluded with gametogonic development principally in the cecal pouches, distal to the cecal necks, with comparatively few gametogonic stages observed in the lower ileum, cecal necks, and rectum. Of the three "cecal" *Eimeria* species infecting turkeys, *E. gallopavonis* never invaded the cecal pouches during endogenous development. The 1st-generation meront of *E. gallopavonis* was the largest to be detected in any *Eimeria* species infecting turkeys (21 μm × 15 μm containing 200–400 merozoites). First generation and all subsequent endogenous stages were found within enterocytes lining the mid-jejunum, ileum, cecal necks, and rectum.

In conclusion, at least three biologically, morphologically, and molecularly distinct *Eimeria* species are associated with coccidiosis affecting the lower intestinal tract and ceca of turkeys. All three species are capable of producing caseous material within the lumen of the infected intestinal tract, but only *E. meleagridis* and *E. adenoeides* are associated with caseous cecal cores. It is hoped that the availability of sequence-based genotyping of biologically characterized lines of each of these species (El-Sherry et al. 2015) will foster the use of homogenous populations of coccidia for studies on biology, taxonomy, pathogenicity, or immunity of these economically important parasites of domestic turkeys.

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Compliance with ethical standards

All experimental manipulations were reviewed and approved by the University of Guelph's Animal Care Committee and complied with the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals (2nd edition).

Conflict of interest statement The authors declare that they have no conflict of interest.

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References

Chapman HD (2008) Coccidiosis in the turkey. Avian Pathol 37:205–223

Clarkson MJ (1958) Life history and pathogenicity of *Eimeria adenoeides* Moore & Brown, 1951, in the turkey poult. Parasitology 48:70–88

Clarkson MJ (1959) The life history and pathogenicity of *Eimeria meleagridis* Tyzzer, 1927, in the turkey poult. Parasitology 49: 519–528

Clarkson MJ (1960) The coccidia of the turkey. Ann Trop Med Parasitol 54:253–257

Cook SM, Higuchi DS, McGowan AL, Schrader JS, Withanage GS, Francis MJ (2010) Polymerase chain reaction-based identity assay for pathogenic turkey *Eimeria*. Avian Dis 54:1152–1156

Doran DJ, Augustine PC (1977) *Eimeria dispersa* and *Eimeria gallopavonis*: infectivity, survival, and development in primary chicken and turkey kidney cell cultures. J Protozool 24:172–176

El-Sherry S, Ogedengbe ME, Hafeez MA, Barta JR (2013) Divergent nuclear 18S rDNA paralogs in a turkey coccidium, *Eimeria meleagrimitis*, complicate molecular systematics and identification. Int J Parasitol 43:679–685

El-Sherry S, Rathinam T, Hafeez MA, Ogedengbe ME, Chapman HD, Barta JR (2014a) Biological re-description of a genetically typed, single oocyst line of the turkey coccidium, *Eimeria meleagrimitis* Tyzzer 1929. Parasitol Res 113:1135–1146

El-Sherry S, Ogedengbe ME, Hafeez MA, Sayf-Al-Din M, Gad N, Barta JR (2014b) Re-description of a genetically typed, single oocyst line of the turkey coccidium, *Eimeria adenoeides* Moore and Brown, 1951. Parasitol Res 113:1–12

El-Sherry S, Ogedengbe ME, Hafeez MA, Sayf-Al-Din M, Gad N, Barta JR (2015) Sequence-based genotyping clarifies conflicting historical morphometric and biological data for 5 *Eimeria* species infecting turkeys. Poult Sci 94:262–272

Farr MM (1964) Life cycle of *Eimeria gallopavonis* Hawkins in the turkey. J Parasitol 50(3 Section 2, Suppl):52

Farr M, Wehr E, Shalkop W (1961) Pathogenicity of *Eimeria gallopavonis*. V J Sci 12:150–151

Hammond DM, Bowman GW, Davis LR, Simms B (1946) The endogenous phase of the life cycle of *Eimeria bovis*. Parasitology 32:409–427

Hawkins PA (1952) Coccidiosis in turkey. Technical Bulletin 226. Michigan State College Agricultural Experiment Station, East Lansing, MI

Hein H (1969) *Eimeria adenoeides* and *E. meleagrimitis*: pathogenic effect in turkey poults. Exp Parasitol 24:163–170

Joyner LP, Norton CC (1972) The drug sensitivity of recently isolated strains of *Eimeria meleagrimitis* and a laboratory strain of *Eimeria adenoeides* in turkeys to robenidene. Res Vet Sci 13:279–281

Matsler PL, Chapman HD (2006) Characterization of a strain of *Eimeria meleagridis* from the turkey. Avian Dis 50:599–604

McDougald LR, Fitz-Coy SH (2003) Coccidiosis. In: Saif YM, Fadly AA, Glisson JR, McDougald LR, Nolan L, Swayne DE (eds) Diseases of poultry, 12th edn. ISU, Ames, pp 1080–1083

Miska KB, Schwarz RS, Jenkins MC, Rathinam T, Chapman HD (2010) Molecular characterization and phylogenetic analysis of *Eimeria* from turkeys and game birds: implications for evolutionary relationships in Galliform birds. J Parasitol 96:982–986. https://doi.org/10.1645/GE-2344.1

Moore EN, Brown JA (1951) A new coccidium pathogenic for turkeys, *Eimeria adenoeides* n. sp. (Protozoa: Eimeriidae). Cornell Vet 41: 124–135

Ogedengbe ME, El-Sherry S, Whale J, Barta JR (2014) Complete mitochondrial genome sequences from five *Eimeria* species (Apicomplexa; Coccidia; Eimeriidae) infecting domestic turkeys. Parasit Vectors 7:335. https://doi.org/10.1186/1756-3305-7-335

Poplstein M, Vrba V (2011) Description of the two strains of turkey coccidia *Eimeria adenoeides* with remarkable morphological variability. Parasitology 138:1211–1216

Rampin T, Manarolla G, Recordati C, Sironi G (2006) Caecal coccidiosis in commercial male turkeys. Ital J Anim Sci 5:315–317

Remmler O, McGregor JK (1964) A method to facilitate isolation of single coccidial oocysts. J Parasitol 50:294

Trees AJ (2002) Parasitic diseases. In: Jordan FTW (ed) Poultry diseases. Baillière Tindal, London, p p413

Tyzzer EE (1929) Coccidiosis in gallinaceous birds. Am J Hyg 10:269–383

Tyzzer EE, Theiler H, Jones EE (1932) Coccidiosis in gallinaceous birds. II. A comparative study of species of *Eimeria* of the chicken. Am J Hyg 15:319–393

Vrba V, Pakandl M (2014) Coccidia of turkey: from isolation, characterisation and comparison to molecular phylogeny and molecular diagnostics. Int J Parasitol 44(13):985–1000. https://doi.org/10.1016/j.ijpara.2014.06.004

Wehr EE, Farr MM, Shalkop WT (1962) Studies on pathogenicity of *Eimeria gallopavonis* to turkeys. J Protozool 9:8–9

Williams RB (2010) The correct date of the original description and availability of the coccidian name *Eimeria meleagridis* Tyzzer (Apicomplexa: Eimeriorina: Eimeriidae). Syst Parasitol 76:77–79