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Prevalence, infectivity and oocyst sporulation time of rabbit-coccidia in Taiwan

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Abstract. Prevalence of *Eimeria* species parasitizing rabbits (*Oryctolagus cuniculus*) in Taiwan were investigated. Six *Eimeria* species, namely: *Eimeria media* (prevalence, 158/642; 24.6%), *Eimeria magna* (101/642; 15.7%), Eimeria *perforans* (58/642; 9.0%), *Eimeria coecicola* (46/642; 7.2%), *Eimeria piriformis* (16/642; 2.5%), and *Eimeria exigua* (9/642; 1.4%) were observed. The overall prevalence of these coccidial infections in rabbits from pet shops and farms was 46.2% and 41.7%, respectively. Concurrent infections involving 2 or 3 species were often observed, while quadruple-infection was rare. Significant differences ($p < 0.005$) in prevalence were observed between the adult and juvenile rabbits. The minimum time required for oocyst sporulation of *E. media*, *E. piriformis*, *E. magna*, *E. perforans*, *E. exigua*, *E. coecicola* were 10, 20, 32, 12, 16, and 36 hr, respectively. This is the first report on the prevalence of *Eimeria* intestinal infection in commercial domestic rabbits in Taiwan. We demonstrated that these rabbit-infecting *Eimeria* species have high biopotential in that the ingestion of a single sporulated oocyst could successfully produce patent infection in a rabbit. In addition, they also possess high host specificity in that they could not infect mice, golden hamsters, Mongolian gerbils, rats, and guinea pigs.

INTRODUCTION

Rabbit coccidian are obligatory intracellular parasites causing coccidiosis in commercial and domestic rabbitries (Peeters *et al.*, 1981; Taylor *et al.*, 2007). Thus far, 15 species of *Eimeria* in rabbit have been identified. *Eimeria stiedai* is the only one that invades the liver. The other species, namely, E*imeria perforans*, *Eimeria piriformis*, *Eimeria exigua*, *Eimeria media*, *Eimeria magna*, *Eimeria coecicola*, *Eimeria vejdovskyi*, *Eimeria flavescens*, *Eimeria roobroucki*, *Eimeria intestinalis*, *Eimeria agnosta*, *Eimeria nagpurensis*, *Eimeria irresidua*, *Eimeria matsubayashi*, and *Eimeria oryctolagi*, parasitize the small intestine. These can be differentiated by the morphology of oocysts, sporulation time, and prepatent period (Baker, 2007; Taylor *et al.*, 2007). They can cause weight loss or failure to gain weight; diarrhoea, which can be watery and bloody; dehydration; and death, thus leading also to economic losses.

We describe herein the morphometry of *Eimeria* sporulated oocysts and their prevalence in rabbits from commercial rabbit farms and pet shops. Furthermore, we also tested the infectivity of these *Eimeria* species in mice, golden hamsters, Mongolian gerbils, rats, and guinea pigs.

MATERIALS AND METHODS

Faecal Samples

A total of 642 rabbit faecal samples were

collected from 11 districts in northern, central, southern, and eastern Taiwan. Of these samples, 324 were obtained from 15 rabbit farms and 318 from 106 pet shops. The 11 districts were Taipei, Taoyuan, Hsinchu, and Miaoli in northern Taiwan; Taichung, Changhua, and Nantou in central Taiwan; Yunlin and Chiayi in southern Taiwan; Yilan and Hualien in eastern Taiwan.

A modified version of Sheather's sugar flotation technique was used to collect and purify the oocyst from the faeces for morphometric study (Li & Ooi, 2008). Then, the oocysts were incubated in 2.5% potassium dichromate at 25ºC, with gentle shaking on a horizontal shaker at 200 rpm for sporulation by assessing under a light microscope at a magnification of 600x. The degree of sporulation was recorded every 4 hr following incubation in 2.5% potassium dichromate.

Purification of *Eimeria* species oocyst

Oocysts were purified by performing a serial dilution with distilled water until a single sporulated oocyst was obtained in 10 µl solution. Each coccidium-free rabbit was infected with 1 sporulated oocyst via oral inoculation. The OPG (oocyst per gram of faeces) was evaluated daily after the infection until the value was decreased to zero. Faecal samples containing pure strain of the *Eimeria* species were collected and used to passage into other coccidium-free rabbits to obtain a further larger number of oocysts. The purified *Eimeria* species oocysts were then stored at 4ºC until used.

Identification of *Eimeria* species

Eimeria species were identified based on the clinical signs, prepatent period, and oocyst morphology (Baker, 2007; Taylor *et al.*, 2007). The morphological features of the sporulated oocysts, including the size of the oocysts, the presence or absence of residual bodies of the oocyst, the size of the sporocysts, the presence or absence of residual bodies of sporocysts, and the presence or absence of Stieda body were also used to identify the *Eimeria* species.

Experimental animals

Six-week-old New Zealand white male rabbits obtained from a rabbit farm in central Taiwan were orally administered an anticoccidial drug at a concentration of 4g/L (containing 1.5g sulfaquinolaline and 4 g sulfamethazine in 10 g of the drug; Yong Shin Pharmaceutical Co., Taichung, Taiwan) to treat any existing coccidial infections that may have been present. Following this, faecal examinations were carried out daily for 21 days to confirm the absence of *Eimeria* oocysts prior to the initiation of the experiment. The rabbits for which this was confirmed were then designated as "coccidium-free" rabbits. Other experimental animals used in our study including 19 ICR mice, 19 golden hamsters, 19 Mongolian gerbils, 19 Wistar rats, and 19 guinea pigs, all of which were 6 weeks old. For each animal species, 3 animals were individually infected with $1x10^4$ sporulated oocysts of *E. magna*, *E. piriformis*, *E. perforans*, *E. media*, *E. exigua*, or *E. coecicola*, respectively, and 1 animal was uninfected as a control. The experimental animals were obtained from the Laboratory Animal Center of National Taiwan University College of Medicine. All the animals were housed under standard conditions with regard to light and temperature. They were provided water and pellet feed *ad lib*. The animals were cared for and handled according to the rules and regulations prescribed by the Institutional Animal Care and Use Committee (IACUC) of Central Taiwan University of Science and Technology.

Statistical analysis

Data were analyzed by using the χ^2 test for pairwise differences. Differences with a p value of <0.05 were considered significant. The χ^2 test was performed using the SPSS statistical software.

RESULTS

The prevalence of *Eimeria* infection among the rabbits examined was 46.2% (147/318) for the animals that had been

procured from pet shops and 41.7% (135/324) for those obtained from rabbit farms (Table 1). Significant differences ($p < 0.005$) in prevalence were observed between the adult and juvenile rabbits. However, no such significant difference was seen between the male and female rabbits ($p > 0.1$; Table 2). Six species of *Eimeria*, namely, *E. media* (prevalence 24.6%; Fig. 1a), *E. magna* (15.7%, Fig. 1b), E. *perforans* (9.0%, Fig. 1c), *E. coecicola* (7.2%, Fig. 1d), *E. piriformis* (2.5%, Fig. 1e), and *E. exigua* (1.4%, Fig. 1f) were observed among the rabbits surveyed (Table 3). Concurrent infections observed included those involving *E. media* and *E. piriformis*; *E. media* and *E. magna*; *E. magna* and *E. coecicola*; *E. media* and *E. perforans*; and *E. media*, *E. perforans*, and *E. piriformis* (Table 4).

The morphometric measurements of the oocysts of these 6 *Eimeria* species obtained from the infected rabbits are shown in Table 5. The rats, mice, Mongolian gerbils, hamsters, and guinea pigs that had been administered *Eimeria* oocysts were not infected at all by the protozoan. No *Eimeria* oocyst was recovered from the 5 above mentioned animal species up to 14 DPI. However, oocyst wall could be observed in the faeces of these animals at 2 DPI. Aliquots of the oocyst suspensions of all the 6 *Eimeria* species used in the rodent experiment were orally administered to control rabbits, and these animals subsequently developed patent infections. In a rabbit that was infected with a single *E. piriformis* oocyst, an OPG as high as $6x10^5$ at 12 DPI, was observed. This reflected the high proliferative capacity of the protozoan.

DISCUSSION

We observed that when rats, mice, Mongolian gerbils, hamsters, and guinea pigs were orally inoculated with rabbit-derived *Eimeria* species, the parasites failed to infect these animals. This indicates that the protozoa are highly host specific. This observation is significant from a public health viewpoint because in many zoos, theme parks and certain children playgrounds, there is an enclosure set up for children to be able to interact or touch animals like rabbits, guinea pigs or even Mongolian gerbils. Since the *Eimeria* species of rabbit are highly host specific, there is little chance that human or the guinea pigs as well as other rodents that are kept together with the infected rabbits will become infected.

On the other hand, a single sporulated oocyst of the *Eimeria* species could successfully infect a rabbit. This indicates

Table 1. Prevalence of oocysts of *Eimeria* species in faecal samples obtained from 318 rabbits in 106 pet shops and 324 rabbits in 15 rabbit farms in Taiwan

Sampling areas	No.		No. of faecal samples containing *Eimeria* species oocyst / No. examined (% Prevalence)	
	Pet shop	Farm	Pet shop	Farm
Northern	25	0[a]	34/75 (45.3%)	0[a]
Central	55	6	55/165 (33.3%)	82/148 (55.4%)
Southern	17	9	36/51 (70.6%)	53/176 (30.1%)
Eastern	9	0[a]	22/27 (81.5%)	0[a]
Total	106	15	147/318 (46.2%)	135/324 (41.7%)

[a] No rabbit farm was found in northern and eastern Taiwan

Table 2. Prevalence of oocysts of *Eimeria* species in faecal samples of different age and sex of rabbits in Taiwan

Age	Sex	No. of faecal samples	Positive infection with *Eimeria* species	Prevalence of *Eimeria* species (%)		
Adult rabbits (age ≥ 4 mo[b])	Male	93	26	26/93 (28.0)	58/186 (31.2 [a])	135/324 (41.7)
	Female	93	32	32/93 (34.4)		
Juvenile rabbits (age < 4 mo[a])	Male	72	40	40/72 (55.6)	77/138 (55.8 [a])	
	Female	66	37	37/66 (56.1)		
Total		324	135			

Most fecal samples obtained from pet shops were collected from juvenile rabbits. No comparison was made between adult and juvenile or male and female rabbits obtained from pet shops
[a] Significant differences ($p < 0.005$). [b] mo: month

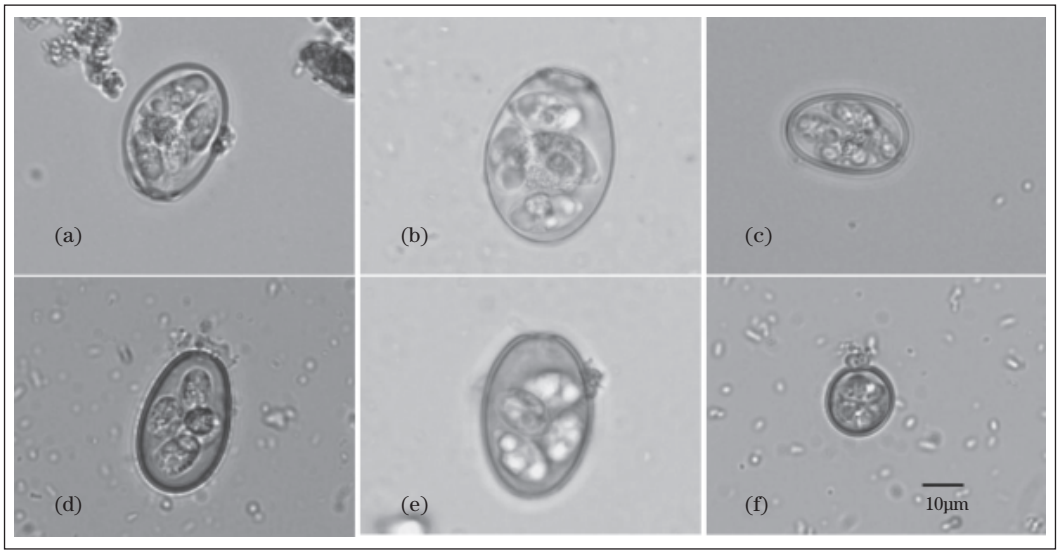

Figure 1. Sporulated oocyst of *Eimeria* spp. (a) *Eimeria media.* (b) *Eimeria magna*. (c) *Eimeria perforans.* (d) *Eimeria coecicola.* (e) *Eimeria piriformis.* (f) *Eimeria exigua.* Bar = 10 µm

Table 3. Prevalence of *Eimeria* spp. from 282 positive faecal samples of rabbits in Taiwan

Infection	Numbers of Pet shop	Numbers of Farm	Prevalence (%)
Single-infection	80	96	176/282 (62.4)
Dual-infection	57	34	91/282 (32.3)
Triple-infection	9	4	13/282 (4.6)
Quadraple-infection	1	1	2/282 (0.7)
Total	147	135	282

Table 4. Prevalence of *Eimeria s*pp. from 642 rabbit faecal samples

Species	Pet shop	Farm	Prevalence (%)
E. media	98	60	158/642 (24.6)
E. magna	63	38	101/642 (15.7)
E. perforans	31	27	58/642 (9.0)
E. coecicola	22	24	46/642 (7.2)
E. piriformis	8	8	16/642 (2.5)
E. exigua	3	6	9/642 (1.4)

Table 5. Morphometric measurements and characteristics of *Eimeria* species isolated from rabbits

Species	L x W of oocyst (µm)	L x W of sporocyst (µm)	L x W of sporozoite (µm)	OR	SR	SB	Sporulation time in hr[a] (95% sporulated)	
							Our study in hr	Taylor et al., 2007 (days)
Eimeria media	29.3 x 18.3 (21.8–34.6 x 16.0–21.3)	13.8 x 7.6 (8.0-17.3 x 5.3–10.6)	9.7 x 3.9 (6.7–12.0 x 2.7–5.3)	+	+	+	10 (16)	2
Eimeria magna	36.6 x 27.6 (31.9–39.9 x 22.6–34.6)	15.2 x 8.8 (10.6–17.3 x 8.0–10.6)	10.2 x 5.0 (6.7–12.0 x 4.0–6.7)	+	+	–	32 (44)	2–3
Eimeria perforans	20.9 x 13.0 (16.0–29.3 x 8.0–19.6)	8.6 x 4.1 (5.3–12.0 x 2.7–6.7)	6.9 x 3.4 (3.2–9.2 x 2.3–4.2)	+	+	+	12 (16)	1.5–2
Eimeria coecicola	33.4 x 21.8 (33.0–36.2 x 20.1–24.3)	12.7 x 7.4 (9.5–15.9 x 6.4–9.5)	7.4 x 4.2 (5.3–9.0 x 3.0–5.5)	+	+	+	36 (48)	4
Eimeria piriformis	29.3 x 20.0 (26.6–34.6 x 15.9–23.9)	14.0 x 7.3 (8.0–16.0 x 4.0–9.3)	8.8 x 4.5 (8.0–13.3 x 2.9–7.2)	–	+	+	20 (27)	4
Eimeria exigua	17.1 x 13.6 (13.3–21.3 x 10.6–18.6)	7.1 x 4.7 (5.3–8.0 x 2.7–5.3)	4.8 x 2.5 (3.3–6.2 x 1.8–3.4)	–	+	–	16 (36)	1

Average of 100 oocysts measured. L: length; W: width; OR: oocyst residuum; SR: sporocyst residuum; SB: Stieda body; [a]First sporulated oocyst observed

not only the high host-specificity but also a very high biopotential of the rabbit-infecting *Eimeria* species.

Rabbits with high parasitic burden had been observed to shed faeces containing occult blood (Li & Ooi, 2009), albeit being demonstrated only for *E. perforans* infection. This indicates that the intestinal *Eimeria* infections can potentially cause haemorrhage and thus are quite detrimental to the health of the rabbit host.

Although many reports on survey of *Eimeria* species infections in rabbit have been published (Fenerich *et al.*, 1973; Catchpole & Norton, 1979; Peeters *et al.*, 1981; Kasim & Al-Shawa, 1987; Polozowski, 1993; Gres et al., 2003). Our present study is the first report on the prevalence of

Eimeria species infections in rabbits in Taiwan. Despite that *E. stiedai* infection was not observed in any of the rabbits examined, occasional occurrences of this infection in pet rabbits brought to veterinary hospitals in Taiwan have been reported (Wang & Tsai, 1991).

Moreover, we report here for the first time the time required for oocyst sporulation in the 6 rabbit-infecting *Eimeria* species under a standard technique. We found the sporulation time of these 6 species to be shorter than those previously published (Ryley & Robinson, 1976; Taylor *et al.*, 2007). However, the oocyst and sporocyst morphology of the 6 *Eimeria* species examined in our study were identical to those described previously. Determining the sporulation time of the various *Eimeria* species can be useful for programme to control infections by these species in rabbits.

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