NOTE *Parasitology*

Survey of Coccidial Oocysts and Parasite Eggs in Feces of Free-Ranging *Grus japonensis*

Takao SARASHINA[1], Yuji UZUKA[2], Shigeyuki TANABE[1], Yuzaburo OKU[3], Yuki WATANABE[4], Nobumichi KUROSAWA[5] and Masakazu NISHIMURA[1]

[1]*School of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555,* [2]*Course of Veterinary Medicine, Faculty of Applied Biological Science, Gifu University, Gifu 501–1193,* [3]*School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido 060–0818,* [4]*Akan International Crane Center, Aksan-gun, Hokkaido 085–0245 and* [5]*Kushiro NOSAI, Kamikawa-gun, Hokkaido 088–3213, Japan*

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ABSTRACT. Fecal survey of *Eimeria* oocysts and parasite eggs was conducted for 219 fecal samples of free-ranging *Grus japonensis* in Kushiro district in Hokkaido in April 2003. Positive rate and mean oocysts (or eggs) per gram in positive samples were 26% (57/219) and 8.8 (0.2–136) in oocysts of *Eimeria reichenowi*, 18.3% (40/219) and 320 (100–1,000) in trematode eggs, 0.1% (2/219) and 0.2 (0.2–0.3) in eggs of Nematoda A, and 4.1% (9/219) and 0.8 (0.2–3.6) in eggs of Nematoda B, respectively.

KEY WORDS: *Eimeria reichenowi*, *Grus japonensis*, parasite egg.

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According to a report of the Japanese Ministry of Environment in 2002, the population of *Grus japonensis* (Red-crowned crane) increased from a minimum of 33 in 1952 to a maximum of 908 in 2002. The reason for the increase in the population of cranes in 2002 is thought to be good growth conditions due to high temperatures and little rainfall. The number of juveniles in 2002 (115) was the highest number since the record number in 1998. The ratio of juveniles in the 908 cranes in 2002 was 12.1%, which was higher than the ratio in the previous year (10.1%), suggesting that the breeding conditions in 2002 were better than those in previous year.

Disseminated visceral coccidiosis (DVC), a fulminant systemic infectious disease with a high mortality rate, is known to occur in cranes. Experimentally reproduced DVC [3, 10, 11] has been reported in *Grus americana* and *G. canadensis* in north America [1, 2, 10, 11, 12]. In Japanese facilities, DVC has been reported in *G. vipio* and *G. japonensis* in Saitama Prefecture [7, 15], in *G. montana* in Kagoshima Prefecture [14] and in *G. japonensis* in Hokkaido [16] and Hyogo Prefecture [9].

According to Watanabe *et al.* [16], DVC occurred in 3 chicks of Red-crowned cranes fed in the Akan International Crane Center in Hokkaido 2002. The chicks developed temporal diarrhea, anemia, serious hepatitis, bronchitis and pneumonia, but they recovered after two-month treatments. Survey of *Eimeria* oocysts was conducted since infection with DVC was thought to occur under crowding conditions in wintering grounds accompanying the increase in the population of Red-crowned cranes.

Red-crowned cranes in Hokkaido begin to move toward Akan-cho, Tsurui-mura and Onbetsu-cho as wintering grounds from October. Most of the cranes winter in these 3 grounds, and begin to move as pairs for breeding to many wetlands distributed in eastern Hokkaido from January to April next year [8]. A total of 219 crane fecal samples were collected on April 5, 2003 at Shimosetsuri, Tsurui-mura, Akan-gun, Hokkaido, a feeding station known as "Tsurumidai". The Wisconsin sugar flotation method (10 g) was used for counting *Eimeria* oocysts and nematode eggs. Using this method, 1/5 g of oocysts (or eggs) per gram of feces (OPG or EPG) is enable to count as the limit of numeration of OPG (or EPG) theorically. Stall's egg counting method (3 g) was used for trematode eggs and the least number of EPG is 100/g of feces.

Eimeria oocysts were spherical to ovoid-ellipsoid in shape and brownish-yellow in color and sporont had a nucleus (Fig. 1). Oocyst size was the mean value of 100 samples. Oocysts were 10.4–30.4 × 10.4–30.1 (mean: 16.3 × 15.7) μm in size, and the L/W ratio was 1.0–1.2 (mean: 1.0) (Table 1). The positive rate of *Eimeria* oocysts was 26% (57/219), and OPG in positive samples was 0.2–136.3 (mean: 8.8) (Table 2).

Large trematode eggs were yellowish-brown in color, and the embryo was located near the operculum. Egg size was the mean value of 80 samples. Eggs were 94.4–140.8 × 60.8–76.8 (mean: 120.2 × 69.4) μm in size, and the L/W ratio was 1.4–2.0 (mean:1.7) (Table 1). Positive rate of trematode eggs was 18.3 (40/219), and EPG of positive samples was 100–1,000 (mean:320.0) (Table 2).

Thick-shelled eggs of Nematoda A with a plug at each end were small-sized, greenish-brown in color, ellipsoidal, slightly asymmetrical in shape and unembryonated. Egg size was the mean value of 2 samples. Eggs were 56.0–57.6 × 6.0–17.6 (mean: 56.8 × 16.8) μm in size, and the L/W ratio was 3.2–3.6 (mean:3.4) (Table 1). Positive rate of eggs of Nematoda A was 0.1% (2/219), and EPG of positive samples was 0.2–0.3 (mean:0.2) (Table 2).

Small eggs of Nematoda B were colorless, ellipsoidal in shape, thin-shelled and unembryonated. Egg size was the mean value of 20 samples. Eggs were 53.6–70.9 × 26.4–33.6 (mean : 62.0 × 28.5) μm in size, and the L/W ratio was

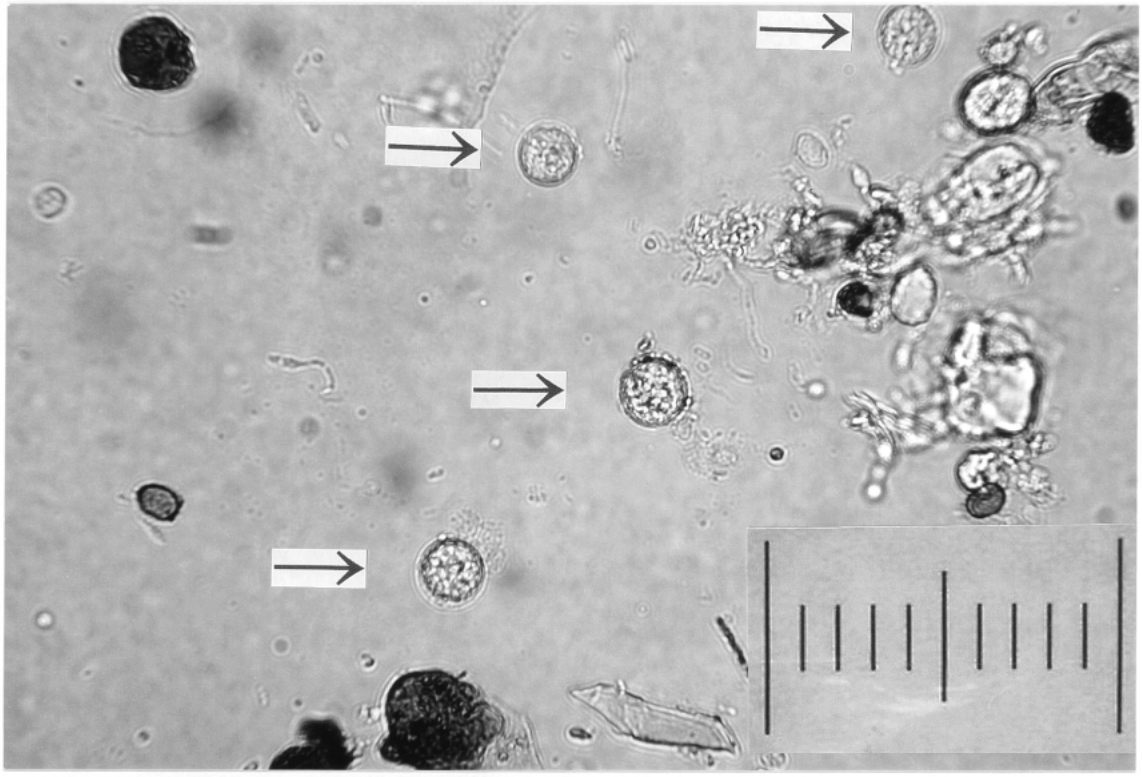

Fig. 1. Unsporulated *Eimeria* oocysts (arrows). Brownish-yellow oocysts were round to ovoid-ellipsoid and sporont had a nucleus. Scale is 100 μm divided every 10 μm.

Table 1. *Eimeria* oocysts, trematode and nematode eggs detected in feces of Red-crowned cranes

Oocysts eggs		*Eimeria* oocysts	Trematode eggs	Eggs of Nematoda A	Eggs of Nematoda B
No. of Samples		100	80	2	20
L[a)] (μm)	Mean ± SD[c)]	16.3 ± 3.6	120.2 ± 7.7	56.8 ± 1.1	62.0 ± 5.6
Range		10.4–30.4	94.4–140.8	56.0–57.6	53.6–70.9
W[b)] (μm)	Mean ± SD	15.7 ± 3.6	69.4 ± 3.7	16.8 ± 1.1	28.5 ± 1.7
Range		10.4–30.1	60.8–76.8	16.0–17.6	26.4–33.6
L/W ratio	Mean	1.0	1.7	3.4	2.2
Range		1.0–1.2	1.4–2.0	3.2–3.6	1.7–2.6

a) L: length.
b) W: width.
c) SD: standard deviation.

1.7–2.6 (mean:2.2) (Table 1). Positive rate of eggs of Nematoda B was 4.1% (9/219), and EPG of positive samples was 0.2–3.6 (mean:0.8) (Table 2).

Eimeria oocysts were spherical to ovoid-ellipsoid in shape and brownish-yellow in color and sporont had a nucleus. *Eimeria* oocysts (n=100) were 10.4–30.4 × 10.4–30.1 (mean:16.3 × 15.7) μm in size and the L/W ratio was 1.0–1.2 (mean:1.0). Previous reports described that size and the L/W ratio (Courtney *et al.* [5] and Novilla *et al.* [10]) in oocysts (n=100) of *E. reichenowi* were 13–22 × 11–19 (mean:17.8 × 15.3) μm, 1.16 and 15–25 × 13–18 (mean:18.5 × 16.1)μm, 1.15, respectively. Consequently, *Eimeria* oocysts found in this study were identified morphologically as *E. reichenowi*.

OPG in positive samples was 0.2–136.3 (mean: 8.8). The reason for present low levels in OPG is thought to be due to the limitation of the Wisconsin method.

Both *E. gruis* and *E. reichenowi* have been always been

Table 2. Positive rate, oocysts (or eggs) per gram of feces of *Eimeria* oocysts, trematode and nematode eggs detected in feces of Red-crowned cranes

Oocysts eggs		*Eimeria* oocysts	Trematode eggs	Eggs of Nematoda A	Eggs of Nematoda B
Positive No. /No. of samples		57/219	40/219	2/219	9/219
Positive rate (%)		26.0	18.3	0.1	4.1
OPG[a)] of positive samples	Mean	8.8			
Range		0.2–136.3			
EPG[b)] of positive samples	Mean ± SD[c)]		320.0 ± 257.4	0.2 ± 0.1	0.8 ± 1.1
Range			100–1,000	0.2–0.3	0.2–3.6

a) OPG: oocysts per gram of feces.
b) EPG: eggs per gram of feces.
c) SD: standard deviation.

detected in feces of free-ranging cranes with DVC [5, 6, 12]. However, *E. gruis* was not detected in the present study. According to Watanabe *et al.* [16], the positive rates of *Eimeria* oocysts in fecal samples of free-ranging Red-crowned cranes in Hokkaido in December (41– 50%) were higher than the positive rates in January to April (11–21%). The sampling period of crane faces in the present study (April) is therefore thought to be the reason for *E. gruis* not being detected.

The positive rate of *Eimeria* oocysts in April in the present study (26%) was lower than that in December in the study by Watanabe *et al.* [16]. New infection is thought to not develop during the cold winter months when sporulation of oocysts in feces on the ground is not possible. Moreover, it is known that the coccidial life cycle is self-limiting, so that coccidiosis is a self-limiting disease [13]. Consequently, the low positive rate of oocysts in April is thought to be due to not only the low temperature in winter but also the spontaneous disappearance of oocysts following the transitory multiplication stage before December.

At least 9 species of trematodes and 16 species of nematodes have been reported in cranes [4]. In present study, trematode eggs (18.3%), eggs of Nematoda A (0.1%) and eggs of Nematoda B (4.1%) were thought to be eggs of Echinostomatidae, Trichuridae and Acuaridae, respectively. However, identification of those species awaits further studies.

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