Isolation, identification, and clinical impact of coccidiosis in Japanese quail farms in and around Bhubaneswar, Odisha, India

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Abstract
This study was carried out to isolate and identify different *Eimeria* species of Japanese quail, and to study the clinical effect of coccidiosis on these birds. 50 dropping samples were collected from 10 Japanese quail farms in and around Bhubaneswar, Odisha, India, including the central poultry development organization. Dead birds were collected for post mortem and histopathological examinations. Some farms appeared normal, but others showed the clinical signs of coccidiosis. Dead birds in post mortem examination showed enteritis with watery mucoid contents and ballooning in the small intestine and ceca. All collected samples were *Eimeria* positive. Three *Eimeria* species were isolated; *E. bateri* (58%), *E. uzura* (66%), and *E. tsSandai* (42%). Three patterns of infection were observed; single infection with *E. bateri* (16%), and mixed infection by the three species (42%). Results indicated that coccidiosis is one of the most predominant diseases affecting Japanese quail farms, and that effective control and management practices are required to overcome this problem.

Keywords: Japanese quail, coccidiosis, *Eimeria bateri*, *Eimeria uzura*, *Eimeria tsSandai*

1. Introduction
Quail production can be regarded as a branch of the modern poultry industry. Meat and egg production are the most common reasons for raising these birds [1]. Japanese quails (*Coturnix coturnix japonica*) were originated from Asia, North Africa, and Europe. They were an important model for aviculture because of the increased consumption of exotic eggs and meats. They were regarded as a substitute for chicken production [2]. Coccidiosis resulted in mild and nonspecific clinical signs, and the natural infection was regarded as a subclinical infection, but it was regarded as a limiting factor for the quail industry as a result of the endogenous stages of the parasite that associated with intestinal lesions. The endogenous stages found in the small intestine of Japanese quail were assumed to be the developmental stages of *E. bateri* and *E. uzura*, while the species noticed in the ceca was supposed to be *E. tsSandai* [3]. Clinical coccidiosis causes lesions and mortalities, while subclinical one results in loss of performance due to disorder in the intestinal function [4]. The economic impact of coccidiosis is attributed to the reduction in animal production as a depressed growth rate, higher feed conversion ratio, and increased mortality [5]. Coccidiosis is the most predominant parasitic disease in quails [6].

Young Japanese quails experimentally infected with *E. bateri* showed anorexia, depression, mucoid and watery dropping, and that weight means were mildly reduced on day 3 post-infection [7]. Clinical symptoms of *E. tsSandai* were characterized by watery diarrhea on day 4 post-infection. A bloody cecal lesion was found on day 5 to 8 post infection, while bloody dropping was more visible on days 5 and 6 post-infection, and most of the mortalities were seen at this period. A lethargic anemic condition with anorexia was present. These clinical findings were similar to those of *E. tenella* in chickens [8]. Coccidiosis had adverse effects on both egg production and fertility in bobwhite quails [9]. Coccidiosis caused by *E. uzura*, before sexual maturation, had short term and long term effects on reproductive development and performance [10]. Diarrhea was the only observed clinical finding, and the main manifested lesion was cecal ballooning without bloody exudate in the lumen [11].
E. tsunodai was highly pathogenic and caused signs and lesions of cecal enteritis. The process from the production of the first generation schizont to the formation of the oocysts took place within the ceca [12]. E. bateri was the most successful regarding transmission in mixed infections, depending on shedding a greater number of oocysts. The prepatent periods of Eimeria spp. in experimentally inoculated Japanese quails were 4 days for E. bateri, but 5 days for E. uzura and E. tsunodai [2]. Desquamation of intestinal mucosa and cecal necrosis were observed in Japanese quails infected with Eimeria species. Developmental stages of Eimeria particularly merozoites and schizonts were noticed in the intestinal epithelium. Schizonts were found in the caecum accompanied with desquamation and necrosis of epithelial lining [13].

The most common Eimeria species isolated from Japanese quails in Brazil were: E. bateri, E. uzura, E. tsunodai, and E. fluminensis [13]. A 27% total coccidial infection rate was recorded in Japanese quail farms in Egypt [14]. The total coccidial infection rate in Japanese quail farms in Saudi Arabian was 29% [15]. Three Eimeria species were isolated from mosul, Iraq: E. tsunodai (44.8%), E. uzura (34.5%), and E. bateri (24.1%) [16]. Four Eimeria species were recorded in Baghdad: E. bateri (66.11%), E. fluminensis (38.33%), E. tsunodai (45%), and E. uzura (23.88%) [17]. A coccidial infection rate of 40.7% was recorded in Japanese quail farms in Egypt [18].

Coccidiosis is considered as one of the most common diseases affecting Japanese quail farms. Till now there is no report about Japanese quail coccidiosis in Odisha state. Therefore, studies on quail coccidiosis management through diagnosis by clinical diagnosis, and isolation and identification of different Eimeria species are very important issues for the improvement of quail production.

2. Materials and Methods
2.1 Collection of dropping samples
Fifty dropping samples were collected from the litter in plastic screw-capped cups from 10 Japanese quail farms in and around Bhubaneswar, including the central poultry development organization (CPDO), Bhubaneswar, Odisha, India during the period from June 2019 to December 2019. Quails were clinically examined. Samples were sent in the icebox to the laboratory of the Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, and examined by direct smear [19], and by flotation technique under the light microscope (10X and 40X) [20].

2.2 Post mortem examination of dead birds and histopathological examination
Dead birds were collected for post mortem examination and detection of coccidial lesions in the intestine and cecum. Contents and mucosal scrapings were taken from Intestines and caeca for microscopic examination to detect oocysts and developmental stages of Eimeria. Specimens from intestines and caeca were taken in 10% formal saline solution for histopathological examination.

2.3 Isolation and identification of Eimeria from dropping samples
2.3.1 Preparation of samples and oocyst concentration
Dropping samples were collected and emulsified in tap water then passed through a wire mesh screen. The emulsion was left to sediment for 15-20 minutes. The supernatant fluid was discarded gently. Sediment was rewashed several times and centrifuged for 5 minutes at 1500 r.p.m [21].

2.3.2 Sporulation of oocyst
The freshly collected oocysts were suspended in a 2.5% freshly prepared potassium dichromate solution and incubated at room temperature (average 25°C) for few days (3-7 days) for sporulation [22]. In wide Petri dishes, oocysts were incubated with not more than 0.5 cm depth of potassium dichromate solution. Frequent aeration was done by shaking the suspension twice daily for few minutes [23]. The sporulation time of oocysts was calculated.

2.3.3 Harvesting and preservation of sporulated oocysts
Eimeria oocysts were harvested according to the methods described previously [24, 25] then preserved in 2.5% potassium dichromate solution at 4°C.

2.3.4 Samples examination and identification of different Eimeria isolates
Oocysts examination was done by flotation technique and microscopic examination [20]. Identification was done under the light microscope (40X and 100X) beside morphometric identification that was done by calibrated ocular micrometer [27]. For measurements, at least one hundred oocysts were measured. Identification relied on morphological characteristics such as (oocyst shape and size, sporocyst size and shape, presence or absence of microspore, polar granules, oocystic and sporocystic residuum, and stieda body of sporocyst) according to the identification guides described previously [2, 3, 13].

3. Results and Discussion
3.1 Clinical examination of Japanese quail farms and histopathological results
Most of the examined birds appeared normal, but the quails in some farms showed ruffled appearance, thin weak breast muscle, and knife-edged keel bone with dropping was wet coffee-colored or tinged with blood and mucus. Clinical signs were more severe in young age quails than adults. Similarly, young Japanese quails infected by E. bateri showed ruffled appearance, thin weak breast muscle, and wet mucoid dropping [7]. Dead birds in post mortem examination showed enteritis with watery mucoid contents, ballooning in the small intestine, congestion of intestine, and thickened intestinal mucosa (Fig. 1). Similar results were previously reported regarding intestinal coccidiosis in Japanese quail [18]. Japanese quails infected by cecal coccidiosis (E. tsunodai) had thin weak breast muscle, knife-edged keel bone, and wet coffee-colored dropping which sometime was tinged with blood and mucus. In post mortem examination, ballooning (Fig. 2) and watery mucoid contents, sometime tinged with blood, were found in the ceca. Similarly, a lethagic anemic condition with anorexia was observed in Japanese quails infected by E. tsunodai [8]. Diarrhea was the only observed clinical finding, and the main manifested lesion was cecal ballooning without bloody exudate in the lumen [11]. Developmental stages and non-sporulated oocysts of Eimeria were detected in the mucosal scraping of intestines (Fig. 3). Histopathological examination revealed necrosis and desquamation of intestinal mucosal villi with presence of
developmental stages of *Eimeria* accompanied by inflammatory cells infiltration and edema (Fig. 4). Cecum showed necrosis of villous epithelium with presence of developmental stages of *E. tsunodai*, inflammatory cells in the sub-mucosa, and mucus on the mucosal surface (Fig. 5). Similar findings were previously reported [3].

**Fig 1:** Ballooning (black arrow) and congestion (red arrows) in the ileum of Japanese quail infected by intestinal coccidiosis (up), and thickening of intestinal mucosa (blue arrow) (down).

**Fig 2:** Ballooning in the ceca of a Japanese quail infected by cecal coccidiosis (*E. tsunodai*).

**Fig 3:** Ruptured schizonts (white arrows) and a non-sporulated oocyst (black arrow) of *E. bateri* in a direct smear of mucosal scraping from the duodenum of a Japanese quail.

**Fig 4:** Necrosis and desquamation of mucosal villi of small intestine with presence of oocysts (arrows) of *E. bateri* and inflammatory cells infiltration (H&E, 40x).

**Fig 5:** Necrosis of the cecal villous epithelium with presence of few oocysts of *E. tsunodai* and inflammatory cells in the sub-mucosa, and mucus on the mucosal surface (H&E, 40x).
3.2 Prevalence of coccidial infection

All collected samples in our study were *Eimeria* positive. This is supported with that coccidiosis is the most predominant parasitic disease in quails [6]. On the other hand, a 27% total coccidial infection rate was recorded in Japanese quail farms in Egypt [14], and a 29% total coccidial infection rate was reported in Japanese quail farms in Saudi Arabian [15].

This difference in the infection rate may be attributed to the difference in the geographical areas and in the systems of using anticoccidial agents.

Concerning the site of infection, intestinal coccidiosis represented 58% (29/50), cecal coccidiosis (*E. tsunodai*) alone was absent, while infection by both intestinal and cecal species was 42% (21/50) (Table 1).

### Table 1: Infection rate according to the site of infection.

| Site of infection | Only cecal infection (E. tsunodai) | Only intestinal infection (E. bateri and E. uzura) | Mixed infection (cecum + intestine) | Total positive samples |
|-------------------|-----------------------------------|---------------------------------------------------|-----------------------------------|------------------------|
| No. of samples    | 0                                 | 29                                                | 21                                | 50                     |
| Percentage (%)    | 0%                                | 58%                                               | 42%                               |                        |

3.3 Identification of the isolated *Eimeria* species

*Eimeria* isolates were morphologically identified as *E. bateri* (58%), *E. uzura* (86%), and *E. tsunodai* (42%) (Table 2). These species are very common in Japanese quail farms worldwide, and they were isolated and described with similar morphological characters in previous studies [2, 3, 16, 28].

### Table 2: Infection rate of each *Eimeria* species.

| Species      | E. bateri | E. uzura | E. tsunodai | Total positive samples |
|--------------|-----------|----------|-------------|------------------------|
| No. of cases | 29        | 43       | 21          | 50                     |
| Percentage (%) | 58%        | 86%      | 42%         |                        |

3.3.1 *E. bateri*

The sporulated oocysts were ovoidal to ellipsoidal, measured 22-30 µm by 15-21.5 µm with a more common range 25x20 µm and a shape index 1.2-1.5 (1.35). The oocyst was smooth bilayered (colorless outer layer and brownish inner one). One to two refractive polar granules were present. The micropyle and residual body of oocyst were absent. The sporocysts were ovoid, measured 10-13.2 µm by 6.3-8 µm with a more common range (12.5×7.5 µm). They had a nipple like stieda body and a prominent rounded sub stieda body. The sporocyst residual body was present as small granules dispersed among the sporozoites which were in pairs with refractive globules visible in the enlarged extremity (Fig. 6) (Table 3) (Chart 1).

3.3.2 *E. uzura*

The sporulated oocysts were ovoidal to ellipsoidal in shape, measured 18-27.5 µm by 15-20.1 µm with a more common range of 22.8×17.5 and a shape index of 1.1-1.5 (1.3). The oocyst was smooth and bilayered (colorless outer layer and brownish inner one). One to four refractive polar granules were present (sometimes with a massive aspect not refractive). The micropyle and residual body of oocyst were absent. The sporocysts were ovoid to elongate, measured 11-13.9 µm by 5.2-7 µm with a more common range of 12.5×6.25 µm. They had a piriform or knob-like or half-moon shape stieda body and a prominent rounded sub stieda body. The residual body of sporocysts was present as small granules dispersed among sporozoites which were in pairs with refractive globules visible in the enlarged extremity (Fig. 7) (Table 3) (Chart 1).

3.3.3 *E. tsunodai*

The sporulated oocysts were spherical to ellipsoidal in shape, measured 15.2-22.5 µm by 14-18.9 µm with a more common range of 20-15 µm and 1.3 shape index. The oocyst was smooth bi-layered (colorless outer layer and brownish inner one) with one flattened end in some oocysts. One to four refractive polar granules were present. The micropyle and residual body of oocyst were absent. The sporocysts were ovoid, measured 10-12 µm by 5-6 µm with a more common range of 10×5 µm. They had a finer end where a small triangular or nipple-like stieda body projected and a rectangular, barely discernible sub stieda body. The residual body of sporocysts was present as small granules dispersed among sporozoites which were in pairs with refractive globules visible in the enlarged extremity (Fig. 8) (Table 3) (Chart 1).

### Table 3: Morphological characteristics of different isolated *Eimeria* oocysts

| Oocyst           | Shape          | Length(µ) (more common) | Width(µ) (more common) | Shape index (more common) | Polar Granules | Stieda Body | Sub-stieda Body |
|------------------|----------------|-------------------------|------------------------|---------------------------|----------------|-------------|-----------------|
| **E. bateri**    | Ovoidal to ellipsoidal | 22-30 (25)             | 15-21.5 (20)           | 1.2-1.5 (1.35)            | 1-2 refractive | Ovoidal     | Prominent, rounded |
|                  | Sub spherical to ovoidal to ellipsoidal | 18-27.5 (22.8) | 15-20.1 (17.5) | 1.1-1.5 (1.3) | 1-4 refractive, sometimes with massive non-reactive aspect | oval to elongated | 11-13.9 (12.5) | Piriform or knob-like or half-moon |
| **E. tsunodai**  | Spherical to ellipsoidal | 15.2-22.5 (20) | 14-18.9 (15) | 1.3 | 1-4 Refractive | Oval | 10-12 (10) | Small triangular or nipple-like |

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There were three patterns of infection: single infection with *E. bateri* (16% [8/50]), single infection with *E. uzura* (42% [21/50]), and mixed infection by the three species (42% [21/50]) (Table 4).

| Species                  | Single infection with *E. bateri* | Single infection with *E. uzura* | Mixed infection with the 3 species | Total positive samples |
|--------------------------|-----------------------------------|----------------------------------|-----------------------------------|------------------------|
| No. of samples           | 8                                 | 21                               | 21                                | 50                     |
| Percentage (%)           | 16                                 | 42                               | 42                                |                         |

**Table 4: Patterns of infection**

Fig 6: *E. bateri* sporulated oocyst in saturated salt solution at 100x (left), and 40x by calibrated micrometer (right).

Fig 7: *E. uzura* sporulated oocyst in saturated salt solution at 100X (right), and 40X by calibrated micrometer (left).

Fig 8: *E. tsunodai* sporulated oocyst in saturated salt solution at 100X (right), and 40X by calibrated micrometer (left).

Coccidiosis causes a great impact on quail production and invades the Japanese quail farms. Therefore, it is so important to conduct further studies on quail coccidiosis including; biology, epidemiology, immunization, and treatment with different anticoccidial drugs and herbal products.

**5. Conclusion**

Coccidiosis is one of the major problems present in Japanese quail farms. It affects clinically and economically on quail production including weight gain, feed conversion efficacy, and mortalities. The most predominant *Eimeria* species infect Japanese quail were *E. bateri*, *E. uzura* and *E. tsunodai*. The infection severity and rate increased at young age compared to adults. *E. tsunodai* was more pathogenic than *E. bateri* and *E. uzura*. 

Chart 1: Infection rate of different *Eimeria* species.
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