



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(6): 302-307

© 2020 JEZS

Received: 12-09-2020

Accepted: 21-10-2020

Mohamed Alaaeldin Mohamed Elmorsy
Ph.D., Scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India.

Geeta Rani Jena

Assistant Professor, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India

Susen Kumar Panda

Professor and Head, Department of Pathology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India

Akshaya Kumar Kundo

Professor and Head, Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India

Dhirendra Kumar

Scientist, Regional Centre, ICAR-Directorate of Poultry Research Bhubaneswar, Odisha, India

Suryakant Mishra

Principal Scientist, Regional Centre, ICAR-Directorate of Poultry Research, Bhubaneswar, Odisha, India

Santosh Kumar Senapati

Associate Professor, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India

Chinnmaya Majhi

M.V.Sc. Scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India

Santosh Kumar Panda

M.V.Sc. Scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India

Manoranjan Das

Professor and Head, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, CVSc & AH, OUAT, Bhubaneswar, Odisha, India

Corresponding Author:

Mohamed Alaaeldin Mohamed Elmorsy
Ph.D., Scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

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

MA Elmorsy, GR Jena, SK Panda, AK Kundo, D Kumar, SK Mishra, SK Senapati, C Majhi, SK Panda and MR Das

DOI: <https://doi.org/10.22271/j.ento.2020.v8.i6d.7870>

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* (86%), and *E. tsunodai* (42%). Three patterns of infection were observed; single infection with *E. uzura* (42%), 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 tsunodai*

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. tsunodai* [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. tsunodai* 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 [11].

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 floatation 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 ceca for microscopic examination to detect oocysts and developmental stages of *Eimeria*. Specimens from intestines and ceca 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 concentrated by centrifugation 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 [26]. 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 micropyle, polar granules, oocystic and sporocystic residium, 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 lethargic 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].

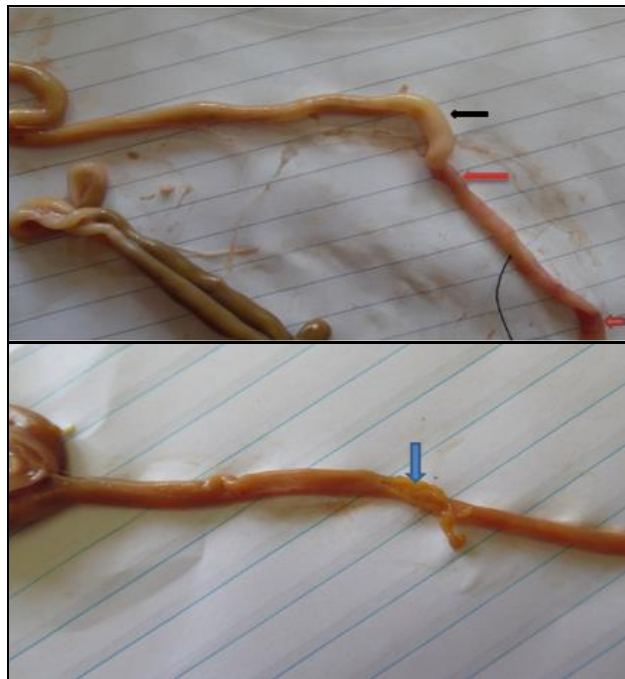


Fig 1: Ballooning (black arrow) and congestion (red arrows) in the ileum of Japanese quail infected by intestinal coccidiosis (up), and thickening of intestinal mucosa (blue arrow) (down).



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

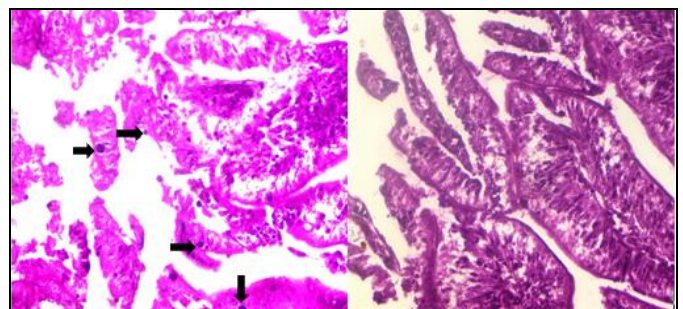


Fig 4: Necrosis and desquamation of mucosal villi of small intestine with presence of oocysts (arrows) of *E. bateri* and inflammatory cells infiltration (H&E, 40x).



Fig 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.

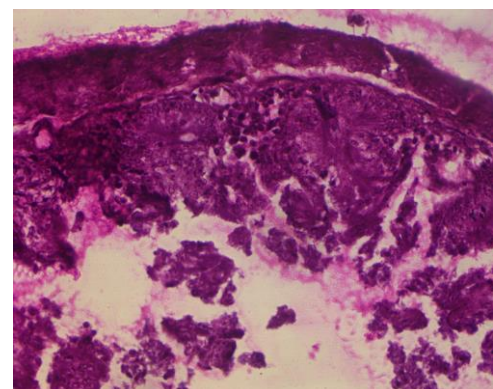


Fig 5: Necrosis of the cecal villous epithelium with presence of few oocysts of *E. tsunodai* and inflammatory cells in the sub-mucosa, and mucus on the mucosal surface (H&E, 40x).

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 the infection rate may be attributed to the difference in the geographical areas and in the systems of using of 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 (<i>E. tsunodai</i>)	Only intestinal infection (<i>E. bateri</i> and <i>E. uzura</i>)	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	<i>E. bateri</i>	<i>E. uzura</i>	<i>E. tsunodai</i>	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 25 \times 20 μm and a shape index 1.2-1.5 (1.35). The oocyst wall 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 \times 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 \times 17.5 and a shape index of 1.1-1.5 (1.3). The oocyst wall 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 \times 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 wall 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 \times 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					Sporocyst				
	Shape	Length(μ) (more common)	Width(μ) (more common)	Shape index (more common)	Polar Granules	Shape	Length(μ) (more common)	width(μ) (more common)	Stieda Body	Sub-stieda Body
<i>E. bateri</i>	Ovoidal to ellipsoidal	22-30 (25)	15-21.5 (20)	1.2-1.5 (1.35)	1-2 refractive	Ovoidal	10-13.2 (12.5)	6.3-8 (7.5)	Nipple-like	Prominent, rounded
<i>E. uzura</i>	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	Ovoidal to elongate	11-13.9 (12.5)	5.2-7 (6.25)	Piriform or knob-like or half-moon	Prominent, rounded
<i>E. tsunodai</i>	Spherical to ellipsoidal	15.2-22.5 (20)	14-18.9 (15)	1.3	1-4 Refractive	Ovoidal	10-12 (10)	5-6 (5)	Small triangular or nipple-like	Rectangular, barely discernible

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).

Table 4: Patterns of infection

Species	Single infection with <i>E. bateri</i>	Single infection with <i>E. uzura</i>	Mixed infection with the 3 species	Total positive samples
No. of samples	8	21	21	50
Percentage (%)	16	42	42	

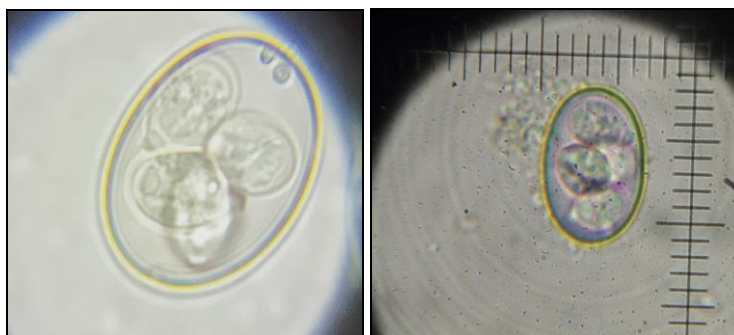


Fig 6: *E. bateri* sporulated oocyst in saturated salt solution at 100x (left), and 40x by calibrated micrometer (right).

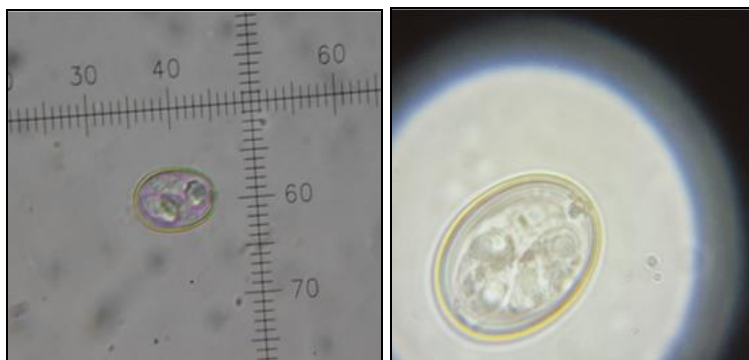


Fig 7: *E. uzura* sporulated oocyst in saturated salt solution at 100X (right), and 40X by calibrated micrometer (left).

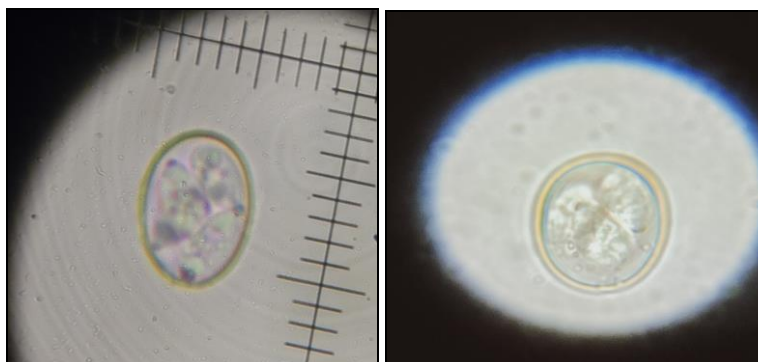


Fig 8: *E. tsunodai* sporulated oocyst in saturated salt solution at 100X (right), and 40X by calibrated micrometer (left).

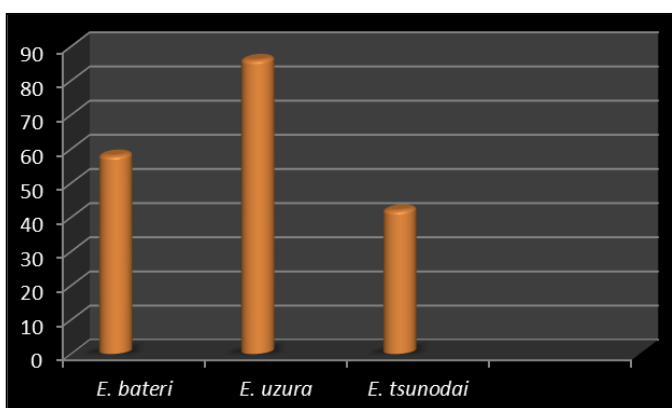


Chart 1: Infection rate of different *Eimeria* species.

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*.

6. Acknowledgement

I need to convey me deepest gratitude to the director of CPDO, Bhubaneswar, Odisha, India for providing facilities for samples collection.

7. References

- Sreeranjini AR, Lyyangar MP, Pramod KD. Histological study on the fibrous architecture of kidney and ureter of Japanese quail (*Coturnix coturnix japonica*). Tamilnadu. Journal of Veterinary and Animal Sciences 2010;6(2):107-110.
- Berto BP, Borba HR, Lima VM, Flausino W, Teixeira-Filho WL, Lopes CWG, et al. *Eimeria* spp. from Japanese quails (*Coturnix japonica*): new characteristic features and diagnostic tools Pesquisa Veterinaria Brasileira 2013;33(12):1441-1447.
- Teixeira M, Teixeira-Filho WL, Lopes CWG. Coccidiosis in Japanese quails (*Coturnix japonica*): Characterization of a Naturally Occurring Infection in a Commercial Rearing Farm, Brazilian Journal of Poultry Science 2004;6(2):129-134.
- Marien M, Gussem DM. Coccidiosis rotation programmes are a must! Poultry World 2007;23(7):34-35.
- Peek HW, Landmanab WJM. Coccidiosis in poultry: anticoccidial products, vaccines and other prevention strategies. Veterinary Quarterly 2011;31(3):143-161.
- Gesek M, Welenc J, Tylicka Z, Otrocka-Domagala I, Paździor K, Rotkiewicz A, et al. Pathomorphological changes in the alimentary system of Japanese quails naturally infected with *Eimeria tsunodai*. Bulletin of the Veterinary Institute in Pulawy 2014;58(1):41-45.
- Norton CC, Peirce MA. The life cycle of *Eimeria bateri* (Protozoa: Eimeriidae) in the Japanese quail (*Coturnix japonica*). The Journal of protozoology 1971;18(1):57-62.
- Tsutsumi Y, Tsunoda K. Pathogenicity of *Eimena tsunodai* from Japanese quails (*Coturnix coturnix japonica*) and susceptibility of the coccidium to some drugs, The Japanese journal of veterinary science 1972;34(3):115-121.
- Ruff MD, Wilkins GC, Chute MB. Prevention of coccidiosis in bobwhites by medication. Poultry science 1987;66(9):1437-1445.
- Ruff MD, Nabi MA, Clarke RN, Mobarak M, Ottinger MA. Effect of coccidiosis on reproductive maturation of male Japanese quail. Avian diseases 1988;32:41-45.
- Umar HA, Lawal IA, Okubanjo OO, Wakawa AM. Morphometric identification, gross and histopathological lesions of *Eimeria* species in Japanese quails (*Coturnix coturnix japonica*) in Zaria, Nigeria. Journal of veterinary medicine 2014, 1-6
- Tsutsumi Y. *Eimeria tsunodai* n. sp. (Protozoa: Eimeriidae). A caecal coccidium of Japanese quails (*Coturnix japonica*). The Japanese journal of veterinary science 1972; 34(1):1-9.
- Teixeira M, Lopes CWG. Species of the genus *Eimeria* (Apicomplexa: Eimeriidae) from Japanese quails (*Coturnix japonica*) in Brazil and *E. fluminensis* for the preoccupied *E. minima* of this quail, Revista Brasileira de Ciências Veterinárias 2002;9(1):53-56.
- Abdel-Hadi DS. Studies on *Eimeria* sp. (Coccidia) infecting quail "*Coturnix japonica*" in Egypt. M.V.Sc. Thesis. Zoology department, Faculty of Science, Cairo University, Bani-suef branch, Bani-suef, Egypt 2008.
- Bashtar AR, Abdel-Ghaffar F, Al-Rasheid KA, Mehlhorn H, Al Nasr I. Light microscopic study on *Eimeria* species infecting Japanese quails reared in Saudi Arabian farms. Parasitology research 2010;107(2):409-416.
- Mohammad NH. A study on the pathological and diagnosis of *Eimeria* species infection in Japanese quail. Basrah Journal of Veterinary Research 2012;11(1):318-333.
- Al-Saeedy HY, AL-Rubaie HM. Epidemiological study of coccidiosis in quail in Baghdad city. Journal of Kerbala University 2014;12(4):42-49.
- Elmorsy MA. Studied on quail coccidiosis. M.V.Sc. Thesis. Poultry and Rabbit Diseases Department, Faculty of Veterinary Science, Mansoura University, Mansoura, Egypt 2017.
- Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. Veterinary Parasitology. 2nd edn, Black Well Science Ltd., Oxford 2003, 276.
- Duszynski DW, Wilber PG. A guideline for the preparation of species descriptions in the *Eimeriidae*. The Journal of parasitology 1997;83:333-336.
- Soulsby E.J.L. Helminthes, arthropods and protozoa of domestic Animals. 6th Edition of of Mönnig's Veterinary helminthology and entomology, Bailliere-Tindall & Cassel Ltd, London, UK 1968.
- Adefolabi TK, Chiejina S. The faecal coccidial oocyst output of adult small ruminants in Nigeria. Nigerian Veterinary Journal 1987;16:1-6.
- Long PL. Maintenance of intestinal protozoa *in vivo* with particular reference to *Eimeria* and *Histomonas*. In: Taylor A, Muller R (Eds.): Isolation and maintenance of Parasites *in vivo*. Blackwell Scientific Publications, Oxford and Edinburgh, United Kingdom 1971, 65-73.
- Shirley MW. *Eimeria* species and strains of chicken. In: Braun R, Eckert J (Eds.): Biotechnology Guidelines on Techniques in Coccidiosis Research. European Commission, Luxembourg 1995, 1-24.
- El-Nahas AF, Awad AM, Abu-Akkada SS. Genetic variation among five Egyptian field isolates of *Eimeria tenella* detected by random amplified polymorphic DNA assay. Global Veterinaria 2011;7(3):256-263.
- Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. Veterinary Parasitology. Longman Sc. & Tech., Harlow, United Kingdom 1987, 150.
- Hendrix CM, Robinson Ed. Diagnostic Parasitology for Veterinary Technicians. 3rd ed, Mosby, Missouri, United States 2006, 232-236.
- El-Morsy MA, Abou El-Azm KI, Awad SS. Efficacy of some anticoccidial drugs on experimentally induced cecal coccidiosis (*E. tsunodai*) in Japanese quails. Egyptian Journal of Veterinary Science 2016;47(2):165-177.