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# Efficacy of Immunization of Japanese Quail (*Coturnix coturnix japonica*) Against the Challenge with Different *Eimeria* Species

M.A. Elmorsy[1,2], M. Das[1], S.K. Senapati[1], G.R. Jena[1], S. Mishra[3], S.K. Panda[1], A.K. Kundu[1], D. Kumar[3]

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## ABSTRACT

**Background:** Coccidiosis is one of the most dangerous diseases infecting Japanese quails. This work was conducted to study the efficacy of immunization of Japanese quail against the challenge with different *Eimeria* species causing coccidiosis and to optimize the dose of live sporulated oocysts that give immunity without adverse effects.

**Methods:** Dropping samples were collected from different Japanese quail pens of the Central Poultry Development Organization, Bhubaneswar, Odisha, India. Isolation, identification, single oocyst isolation and propagation were done for each species. On the 2nd day of age, Japanese quails were divided into 10 groups and orally inoculated with low doses, 100 and 1000 live sporulated oocysts, of each isolated *Eimeria* species separately. Four weeks post-immunization, the quails were challenged with a high dose of the respective species. Immunization efficacy was assessed regarding clinical signs, mortality rate, weight gain, feed consumption, feed conversion ratio, oocysts output, lesion scores and hematological parameters.

**Result:** All collected samples were *Eimeria* positive. Three *Eimeria* species were isolated and identified as *E. bateri, E. uzura* and *E. tsunodai*. Experimental results revealed that the immunized quails, either by 100 or 1000 oocysts of any isolated species, showed better results compared to the challenged, non-immunized controls. The 100-oocysts dose gave better results than those of 1000-oocysts dose without significant differences. These results indicated the effectiveness of immunization as an important tool in the management of Japanese quail coccidiosis.

**Key words:** Coccidiosis, *Eimeria bateri, Eimeria uzura, Eimeria tsunodai*, Immunization, Quail, Vaccination.

## 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 (Sreeranjini *et al.,* 2010).

Coccidiosis was regarded as a limiting factor for the quail industry as a result of the endogenous stages of the parasite those associated with intestinal lesions. The economic impact of coccidiosis is attributed to the reduction in animal production attributes as denoted by depressed growth rate, higher feed conversion ratio and increased mortality (Peek and Landmanab, 2011).

Because of the increasing problem of drug resistance to anticoccidial drugs, the search for anticoccidial vaccines has become an important topic. Therefore, it is necessary to establish a potential standard method for providing protection by live multivalent vaccines against clinical coccidiosis. There is no specific evidence that chickens are immune to coccidiosis other than by challenging with virulent parasites. Therefore, the major consideration in the assessment of vaccine efficacy is the clinical status of the birds following virulent challenge, particularly growth rate and feed conversion ratio (Williams and Catchpole, 2000). Bobwhite quails immunized by 100 and 1000 oocysts of *E. lettyae* provided good protection against challenge concerning various parameters (Gerhold *et al.,* 2010). Attenuation of the virulence of coccidia in the live vaccines is largely done by the size of the dose and by the means of administration (McDougald and Fitz-coy, 2013).

Although many previous studies were conducted on vaccination against coccidiosis of chicken and some *Eimeria* species of bobwhite quail, studies on immunization of Japanese quail against coccidiosis are limited. Thus, the present study was established to determine the efficacy of immunization of Japanese quail by low doses of live sporulated oocysts of each *Eimeria* species separately

[1]College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.
[2]Department of Poultry and Rabbit Diseases, Faculty of Veterinary Medicine, Mansoura University-35516, Egypt.
[3]ICAR-Directorate of Poultry Research, Bhubaneswar-751 003, Odisha, India.

**Corresponding Author:** M.A. Elmorsy, Department of Poultry and Rabbit Diseases, Faculty of Veterinary Medicine, Mansoura University-35516, Egypt. Email: m.alaa@mans.edu.eg

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against a high dose challenge and to optimize the dose that stimulates immunity without adverse effects.

## MATERIALS AND METHODS

### Samples collection and parasite isolation and identification

Dropping samples were collected from the litter of different pens housing different ages of Japanese quail at the Central Poultry Development Organization (CPDO), Bhubaneswar, Odisha, India. Samples packed in icebox were sent to laboratory for examination by direct smear method (Urquhart, 2003) and by floatation technique under the light microscope according to Duszynski and Wilber (1997). Positive samples were prepared and oocysts were collected by concentration according to the method of Soulsby (1968). Freshly collected oocysts were suspended in a 2.5% freshly prepared potassium dichromate solution and incubated for few days (3-7 days) at room temperature for sporulation as described by Long (1971). *Eimeria* oocysts were harvested according to the methods described by Shirley (1995) and preserved in potassium dichromate solution (2.5%) at 4-8°C. Identification was done using light microscope (40X and 100X) besides morphometric identification by calibrated ocular micrometer according to Henddrix and Robinson (2006). Identification was done according to the identification guides described by Berto *et al.* (2013).

### Experimental birds

1-day-old Japanese quail chicks were purchased from CPDO hatchery for isolation and propagation of single *Eimeria* species and for the experimental trial. The quails were kept in the College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India. Quail chicks were kept in brooding cages with their floors covered by a linoleum layer and wood shaving litter in good sanitary conditions to avoid coccidian contamination. Quails received a non-medicated starter ration and ad libitum drinking water. All quails were reared following the directions for the use of experimental animals of the Institutional Animal Ethical Committee of the College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology.

### Single oocyst isolation and propagation

This was done according to the method of Khalafalla and Daugschies (2010) with some modifications. Briefly, clean small petri dishes were divided into very small areas (1×1 $mm^2$) by using a marker pen and marked with different numbers. A first layer of pre-warmed 5% nutrient agar was spread over the divided plates and kept for 10 min at 4°C to solidify. A thin layer of oocysts suspension (100 oocysts / mL) was spread over the first layer. The plates were examined microscopically. A separate single sporulated oocyst was cut from the agar and placed into a small gel capsule, then introduced into the esophagus of 7-days-old Japanese quails followed by few drops of water. Quails were bred in 3 separate groups (one group for each species) to avoid cross-contamination between the different isolates. On day 6 post-infection, intestines and ceca were collected and examined for post mortem lesions. Dropping samples plus intestinal and cecal contents were collected separately and examined by direct smears. The isolated oocysts of different species were compared morphologically with the identification guides previously described by Berto *et al.* (2013) and then preserved for further experimental work. The isolated species were propagated through coccidiosis free Japanese quails, reared in separate sterilized cages to avoid cross-contamination.

### Experimental trial

The experiment was done on January 2021 with a duration of 37 days. It was conducted at the Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology.

On the 2nd day of age, quails were randomly divided into 10 groups of three replicates each, 10 birds per replicate. Birds were treated by oral inoculation (intra esophagus) by a small pipette. Immunization was done on the $2^{nd}$ day of age. It was conducted by oral inoculation (intra esophagus) of 0.2 mL inoculum containing 100 or 1000 oocysts of each *Eimeria* species separately suspended in sterilized distilled water. Challenge was done 4 weeks post-immunization (day 30 of age). It was conducted by oral inoculation (intra esophagus) of 0.5 mL inoculum containing the challenge dose of the respective *Eimeria* species suspended in sterilized distilled water. Quails were grouped as follows:

**Group (1):** Negative control group (non-infected, non-treated): inoculated by a sham-dose of 0.5 mL of sterilized distilled water on day 30 of age.
**Group (2):** *E. bateri* positive control group: Quails were challenged with $1\times10^5$ sporulated oocysts of *E. bateri*.
**Group (3):** Quails were inoculated with 100 sporulated oocysts of *E. bateri* and then challenged with $1\times10^5$ sporulated oocysts of *E. bateri*.
**Group (4):** Quails were inoculated with 1000 sporulated oocysts of *E. bateri* and then challenged with $1\times10^5$ sporulated oocysts of *E. bateri*.
**Group (5):** *E. uzura* positive control group: Quails were challenged with $1\times10^5$ sporulated oocysts of *E. uzura*.
**Group (6):** Quails were inoculated with 100 sporulated oocysts of *E. uzura* and then challenged with $1\times10^5$ sporulated oocysts of *E. uzura*.
**Group (7):** Quails were inoculated with 1000 sporulated oocysts of *E. uzura* and then challenged with $1\times10^5$ sporulated oocysts of *E. uzura*.
**Group (8):** *E. tsunodai* positive control group: Quails were challenged with $4\times10^4$ sporulated oocysts of *E. tsunodai*.
**Group (9):** Quails were inoculated with 100 sporulated oocysts of *E. tsunodai* and then challenged with $4\times10^4$ sporulated oocysts of *E. tsunodai*.

**Group (10):** Quails were inoculated with 1000 sporulated oocysts of *E. tsunodai* and then challenged with $4\times10^4$ sporulated oocysts of *E. tsunodai*.

Regular monitoring of birds was done for clinical signs and mortalities after inoculation and throughout the experiment (37 days). Dropping samples were collected between days 4-9 post-challenge to detect the effect of immunization on oocyst output through counting by McMaster-chamber as described by Conway and Mckenzie (1991).

On days 4, 5, 6 and 7 post-challenge, 3 birds from each group were sacrificed to examine intestines and ceca for lesion scores. The severity of lesions was evaluated according to Elmorsy *et al.* (2021).

Individual blood samples were drawn from each bird via wing vein in EDTA vials on day 7 post-challenge for hematological tests. Calculation of total erythrocyte and total leukocyte counts was done according to the method of Natt and Herrick (1952) and Campbell (1995) by using Natt and Herrick's stain solution. Haemoglobin (Hb) percent was measured by using commercial kit HEMOCOR-D reagent (Crest Biosystems, Goa, India) (cyanmethemoglobin method) as described by Van Kampen and Zijlstra (1961). Packed cell volume (PCV), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the procedures described by Jain (1986). Differential leucocyte count was done through blood smear stained with Wright-Giemsa stain according to the method of Benjamin (1985).

Birds and feed of each replicate were weighed on day 30 of age, prior to challenge and on day 7 post-challenge. Average feed intake, weight gain and feed conversion ratio were calculated.

### Statistical analysis

Data were statistically analyzed by SPSS version 20 by one-way analysis of variance (ANOVA) using least significant difference (LSD) at $P$-value $\leq 0.05$ as described by Snedecor and Cochran (1981).

## RESULTS AND DISCUSSION

### Identification of isolated *Eimeria* species

*Eimeria* isolates were identified as *E. bateri*, *E. uzura* and *E. tsunodai* (Fig 1). The present study revealed that all collected fecal samples were *Eimeria* positive and this agreed with Gesek *et al.* (2014) who reported that coccidiosis is the most predominant parasitic disease in quails. Two *Eimeria* species were isolated from intestine (*E. uzura* and *E. bateri*) and one from cecum (*E. tsunodai*). These results agreed with those of Teixeira *et al.* (2004). The morphological characters of the isolated *Eimeria* species in our study were similar to those previously described by Berto *et al.* (2013).

### Single oocyst isolation and propagation

Single oocyst isolation was successfully carried out for the three identified species. The group inoculated with *E. bateri* showed a mild decrease in the body weight with mucoid and watery diarrhea and this was similar to the findings of Norton and Pierce (1971). Oocysts and lesions were found only in the intestine, represented by ballooning in the duodenum and jejunum with watery intestinal contents (Fig 2).

*E. uzura* inoculated group showed a mild decrease in the body weight with mucoid and watery diarrhea and this agreed with the results of Tsunoda and Muraki (1971) and Ruff *et al.* (1984). Oocysts and lesions were found only in the intestine as ballooning with mucoid watery contents in the duodenum and jejunum, congested parts in jejunum and ballooning in ileum.

*E. tsunodai* inoculated group showed a decrease in body weight, watery diarrhea on day 4 post-inoculation. Dropping was coffee-colored or tinged with blood on days 5 and 6 post-inoculation. These observations were similar to the findings of Tsutsumi and Tsunoda (1972). Oocysts and lesions appeared in the cecum and to some extent in the rectum. Lesions ranged from ballooning in the 2 ceca with foamy watery cecal contents to hardened fibrous cecal contents tinged with blood and thickened cecal mucosa.

### Experimental trial

Positive control groups of each *Eimeria* species were severely affected and showed signs such as depression,

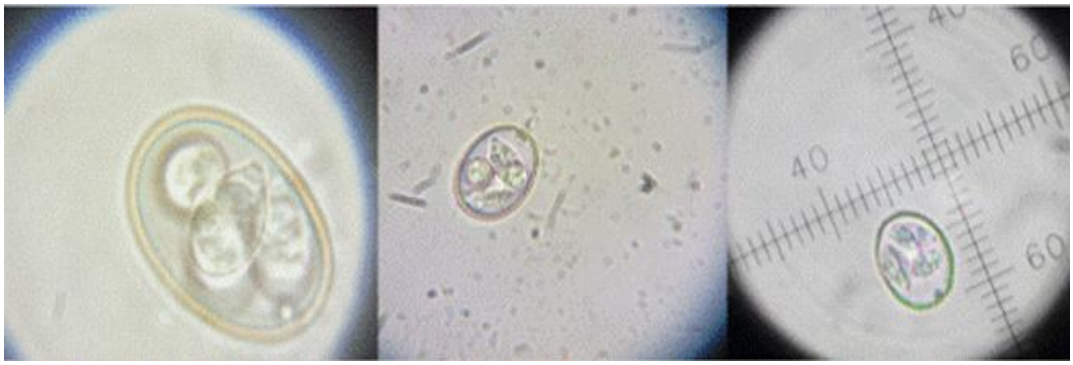


**Fig 1:** *E. bateri* (left) (100X), *E. uzura* (middle) (40X) and *E. tsunodai* (right) (40X) sporulated oocysts in saturated salt solution.

ruffled feathers, huddle together, decrease in appetite and emaciated breast muscle. The fecal abnormalities ranged from soft to brownish foamy mucoid to watery diarrhea. Coffee-colored dropping, blood-tinged dropping and bloody diarrhea were seen in *E. tsunodai* positive control group. The immunized, challenged groups of each *Eimeria* species showed lower clinical signs and fecal abnormalities compared to the non-immunized, challenged groups.

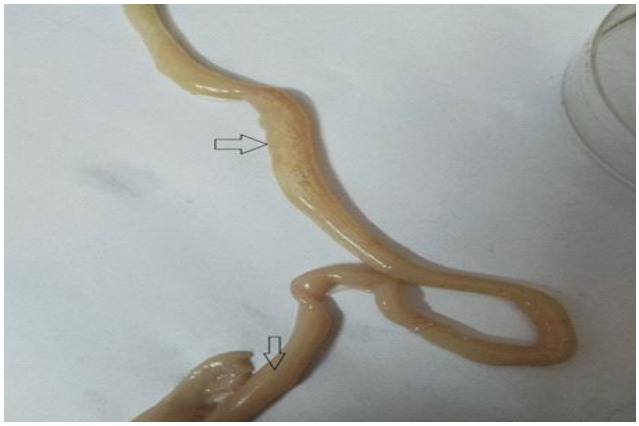

**Fig 2:** Small intestine of a Japanese quail inoculated by *E. bateri*, during propagation, showed ballooning in the duodenum and jejunum (Arrows) with watery and foamy contents (score +2).

The groups challenged with *E. bateri* had no mortalities. *E. uzura* positive control group showed 6.67% mortality, while both 100 and 1000-*E. uzura* oocysts immunized groups had 3.33% mortality. A significant decrease ($P<0.001$) in mortality occurred in 100 and 1000-*E. tsunodai* oocysts immunized groups by 3.33% and 6.67%, respectively, compared to *E. tsunodai* positive control group which possessed the highest mortality percent (20%) (Table 1).

The immunized groups by *E. bateri* or *E. uzura* showed an insignificant decrease ($P>0.05$) in the lesion score compared to that of their positive controls. While, a significant decrease ($P<0.05$) in the lesion score was seen in *E. tsunodai* immunized groups compared to *E. tsunodai* positive control group where bloody cecal core and bloody diarrhea were present. Both 100 and 1000-oocysts immunized groups of each *Eimeria* species showed the same lesion score (Table 1) (Fig 3-9). These observations were in agreement with those reported by Gerhold *et al.* (2010) in Bobwhite quail immunized against *E. lettyae*.

Concerning oocyst output, the immunized groups of each *Eimeria* species showed a dramatic highly significant decrease ($P<0.001$) in the oocyst output compared to their

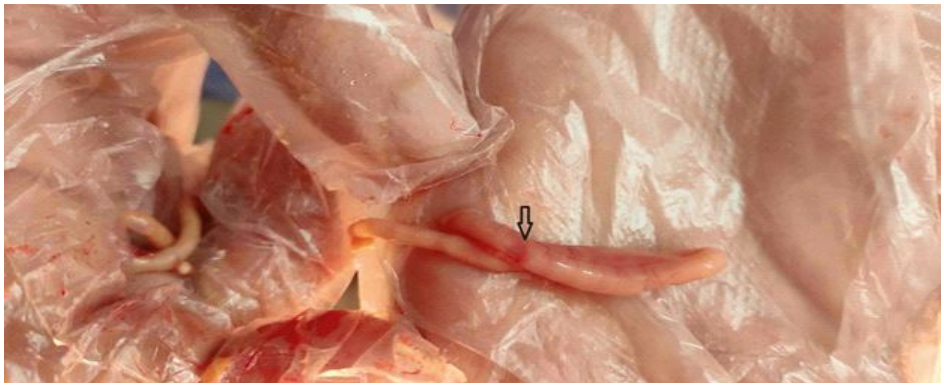

**Fig 3:** Mild congestion (arrow) in the duodenum of a Japanese quail challenged by $10^5$ *E. bateri* oocysts (+ve control group) ($5^{th}$ day post-challenge).

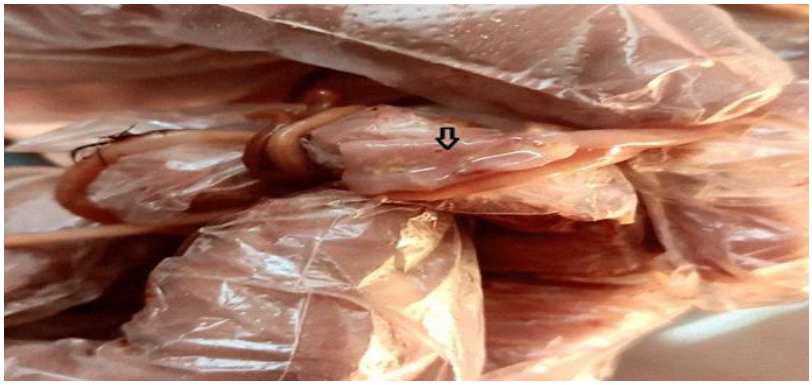

**Fig 4:** Duodenum of a Japanese quail challenged by $10^5$ *E. bateri* oocysts (+ve control group) showed mucoid contents in the lumen with mildly thickened mucosa (arrow) (score +2) ($5^{th}$ day post-challenge).

**Table 1:** Means of mortality rate and lesion score throughout 7 days post-challenge [1].

| | Negative control | *E. bateri* positive control | 100-*E. bateri* oocysts immunized, challenged | 1000-*E. bateri* oocysts immunized, challenged | *E. uzura* positive control | 100-*E. uzura* oocysts immunized, challenged | 1000-*E. uzura* oocysts immunized, challenged | *E. tsunodai* positive control | 100-*E. tsunodai* oocysts immunized, challenged | 1000-*E. tsunodai* oocysts immunized, challenged |
|---|---|---|---|---|---|---|---|---|---|---|
| Mortality | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.67±0.33$^{b}$ | 0.33±0.33$^{b}$ | 0.33±0.33$^{b}$ | 2.00 ±0.00$^{a}$ | 0.33±0.33$^{b}$ | 0.67±0.33$^{b}$ |
| Lesion score | 0.00±0.00$^{c}$ | 1.75±0.25$^{ab}$ | 1.00±0.41$^{b}$ | 1.00±0.41$^{b}$ | 1.75±0.25$^{ab}$ | 1.25±0.25$^{b}$ | 1.25±0.25$^{b}$ | 2.50±0.50$^{a}$ | 1.50±0.29$^{b}$ | 1.50±0.29$^{b}$ |

[1]Means: Mean of 3 replicates / group (Mean ± standard error).

$^{a\text{-}c}$ Means in the same row with different superscripts are significantly different at P≤0.05.

**Table 2:** Means of daily oocyst output, oocyst per gram feces (OPG), 4$^{th}$ - 9$^{th}$ day post-challenge[1].

| | Daily oocyst output (*E. bateri*) | | | Daily oocyst output (*E. uzura*) | | | Daily oocyst output (*E. tsunodai*) | | |
|---|---|---|---|---|---|---|---|---|---|
| Day post challenge | *E. bateri* positive control | 100-*E. bateri* oocysts immunized, challenged | 1000-*E. bateri* oocysts immunized, challenged | *E. uzura* positive control | 100-*E. uzura* oocysts immunized, challenged | 1000-*E. uzura* oocysts immunized, challenged | *E. tsunodai* positive control | 100-*E. tsunodai* oocysts immunized, challenged | 1000-*E. tsunodai* oocysts immunized, challenged |
| 4$^{th}$ | 2851.67±67.72$^{a}$ | 105.93 ±2.60$^{b}$ | 108.93 ±3.54$^{b}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{c}$ |
| 5$^{th}$ | 2314.23±49.29$^{a}$ | 108.60 ± 4.22$^{c}$ | 119.63 ± 2.35$^{c}$ | 1065.33±62.44$^{b}$ | 104.63 ±3.39$^{c}$ | 111.23 ±2.19$^{c}$ | 993.467±46.19$^{b}$ | 96.80 ±1.46$^{c}$ | 97.87 ±1.13$^{c}$ |
| 6$^{th}$ | 1045.17±61.91$^{b}$ | 104.50 ± 3.27$^{c}$ | 112.47 ±2.42$^{c}$ | 1216.97±60.96$^{a}$ | 108.00 ± 3.39$^{c}$ | 113.43 ±2.44$^{c}$ | 971.73 ±59.58$^{b}$ | 98.77 ±0.78$^{c}$ | 101.03 ±1.56$^{c}$ |
| 7$^{th}$ | 839.46±56.09$^{a}$ | 98.10±1.70$^{c}$ | 103.30±3.39$^{c}$ | 790.07±63.54$^{a}$ | 91.37±2.43$^{c}$ | 93.17±4.42$^{c}$ | 627.03±64.71$^{b}$ | 81.20±2.80$^{c}$ | 83.73±5.35$^{c}$ |
| 8$^{th}$ | 672.03± 64.67$^{a}$ | 64.90± 2.95$^{c}$ | 68.13± 3.96$^{c}$ | 628.67±63.52$^{a}$ | 60.77±2.87$^{c}$ | 62.67±3.06$^{c}$ | 501.67±60.71$^{b}$ | 53.07±3.17$^{c}$ | 55.10±3.56$^{c}$ |
| 9$^{th}$ | 380.23±43.73$^{b}$ | 43.97± 2.64$^{d}$ | 45.93±2.53$^{d}$ | 491.73±70.73$^{a}$ | 40.13±1.35$^{d}$ | 42.17±1.59$^{d}$ | 275.80±63.08$^{c}$ | 35.17±2.14$^{d}$ | 35.83±1.30$^{d}$ |

[1] Means: Mean of 3 replicates / group (Mean ± standard error) × 1000.

$^{a\text{-}d}$ Means in the same row with different superscripts are significantly different at P≤0.05.

**Table 3:** Means of feed consumption (g), weight gain (g) and FCR, 7 days post-challenge[1].

| | Feed consumption | Weight gain | FCR |
|---|---|---|---|
| Group (1): Negative control. | 157.69±0.75$^{a}$ | 62.91±1.45$^{a}$ | 2.51±0.05$^{f}$ |
| Group (2): *E. bateri* positive control. | 149.27±1.01$^{ef}$ | 50.04±1.13$^{d}$ | 2.98±0.08$^{c}$ |
| Group (3): 100-*E. bateri* oocysts immunized, challenged. | 153.57±0.92$^{bc}$ | 60.78±1.18$^{a}$ | 2.52±0.06$^{f}$ |
| Group (4): 1000-*E. bateri* oocysts immunized, challenged. | 155.09±1.08$^{b}$ | 57.12±1.14$^{b}$ | 2.71±0.04$^{e}$ |
| Group (5): *E. uzura* positive control group. | 146.88±1.16$^{f}$ | 43.76±0.89$^{e}$ | 3.36±0.08$^{b}$ |
| Group (6): 100-*E. uzura* oocysts immunized, challenged. | 154.00±1.01$^{bc}$ | 55.57±1.30$^{bc}$ | 2.77±0.05$^{de}$ |
| Group (7): 1000-*E. uzura* oocysts immunized, challenged. | 151.92±0.64$^{cd}$ | 52.51±0.93$^{cd}$ | 2.89±0.04$^{cd}$ |
| Group (8): *E. tsunodai* positive control. | 138.77±0.75$^{g}$ | 39.34±0.75$^{f}$ | 3.53±0.05$^{a}$ |
| Group (9): 100-*E. tsunodai* oocysts immunized, challenged. | 150.40±0.89$^{de}$ | 52.21±1.27$^{d}$ | 2.88±0.05$^{cd}$ |
| Group (10): 1000-*E. tsunodai* oocysts immunized, challenged. | 147.69±0.81$^{f}$ | 50.56±0.98$^{d}$ | 2.92±0.04$^{c}$ |

[1] Means: Mean of 3 replicates / group (Mean ± standard error).

$^{a-g}$ Means in the same column with different superscripts are significantly different at P≤0.05.

**Fig 5:** Mild ballooning in the jejunum and ileum (arrows) of a Japanese quail challenged by $10^5$ *E. uzura* oocysts (+ve control group) (score +2) (7$^{th}$ day post- challenge).

**Fig 6:** Ceca of a Japanese quail challenged by $4\times10^4$ *E. tsunodai* sporulated oocysts (+ve control group) showed severe ballooning, hemorrhage and clotted blood causing bloody cecal core (score +3) (6$^{th}$ day post-challenge).

positive control groups. The 100-oocysts immunized groups had oocyst output insignificantly lower (P>0.05) than that of the 1000-oocysts immunized groups (Table 2). Similarly, Gerhold *et al.* (2010) reported that the immunized Bobwhite quail by 100-oocysts of *E. lettyae* produced around 99.7% fewer oocysts compared to the non-immunized, challenged control.

Regarding feed consumption, the immunized groups of each *Eimeria* species showed a significantly better feed consumption compared to their positive controls. The

**Fig 7:** Small intestine of a Japanese quail immunized by *E. bateri* 100-live sporulated oocysts and then challenged by $10^5$ *E. bateri* oocysts, 4 weeks later. All parts of intestine were normal except small parts of ileum (arrow) showed mild ballooning with watery foamy contents (score +1) (6$^{th}$ day post-challenge).

**Fig 8:** Small intestine of Japanese quail immunized by *E. uzura* 100-live sporulated oocyst and then challenged by $10^5$ *E. uzura* oocysts, 4 weeks later. All parts of intestine were normal except small parts of jejunum (arrow) showing mild ballooning with watery foamy contents (score +1) (7$^{th}$ day post-challenge).

difference in feed consumption between the 100 and 1000-oocysts doses of different *Eimeria* species was insignificant (Table 3).

The 100 and 1000-*E. bateri* oocysts immunized groups showed a significant increase (P<0.001) in the weight gain compared to the positive control group. The 100-*E. bateri*

**Table 4:** Means of hematological results, 7 days post-challenge [1].

| Treatments | Hematological parameters 7 days post challenge | | | | | | |
|---|---|---|---|---|---|---|---|
| | RBCs count ($\times10^6$/µL) | WBCs count ($\times10^3$/µL) | Hb (g/dL) | PCV (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) |
| Group (1): Negative control. | 4.42±0.24$^{a}$ | 13.82±1.39$^{b}$ | 13.47±0.32$^{a}$ | 39.93±0.41$^{a}$ | 90.74±4.19$^{a}$ | 30.55±0.99$^{a}$ | 33.71 ±0.45$^{a}$ |
| Group (2): *E. bateri* positive control. | 4.11±0.13$^{ab}$ | 15.33±0.65$^{ab}$ | 12.42±0.31$^{bc}$ | 37.67±0.44$^{c}$ | 95.34±2.93$^{a}$ | 30.25±0.26$^{a}$ | 32.96±0.47$^{abc}$ |
| Group (3): 100-*E. bateri* oocysts immunized, challenged. | 4.31±0.16$^{a}$ | 16.65±0.39$^{a}$ | 13.30±0.26$^{ab}$ | 39.40±0.50$^{ab}$ | 91.51±2.26$^{a}$ | 30.88±0.65$^{a}$ | 33.75 ±0.31$^{a}$ |
| Group (4): 1000-*E. bateri* oocysts immunized, challenged. | 4.24±0.14$^{a}$ | 16.95±0.33$^{a}$ | 13.12±0.28$^{ab}$ | 38.97±0.67$^{abc}$ | 92.26±1.89$^{a}$ | 30.99±0.69$^{a}$ | 33.66±0.28$^{ab}$ |
| Group (5): *E. uzura* positive control. | 3.83±0.34$^{ab}$ | 14.56±0.49$^{b}$ | 11.55±0.37$^{cd}$ | 36.03±0.32$^{d}$ | 95.41±8.03$^{a}$ | 30.45±1.81$^{a}$ | 32.04±0.76$^{bc}$ |
| Group (6): 100-*E. uzura* oocysts immunized, challenged. | 4.17±0.24$^{a}$ | 16.35±0.25$^{a}$ | 12.88±0.37$^{ab}$ | 39.03±0.38$^{abc}$ | 94.23±4.83$^{a}$ | 31.03±0.97$^{a}$ | 32.99±0.63$^{abc}$ |
| Group (7): 1000-*E. uzura* oocysts immunized, challenged. | 4.00±0.24$^{ab}$ | 16.58±.52$^{a}$ | 12.58±0.38$^{ab}$ | 38.28±0.55$^{bc}$ | 96.26±4.73$^{a}$ | 31.57±0.99$^{a}$ | 32.86±0.57$^{abc}$ |
| Group (8): *E. tsunodai* positive control. | 3.53±0.20$^{b}$ | 14.00±0.32$^{b}$ | 10.68±0.37$^{d}$ | 33.87±0.35$^{e}$ | 96.37±4.50$^{a}$ | 30.32±0.74$^{a}$ | 31.53±0.75$^{c}$ |
| Group (9): 100-*E. tsunodai* oocysts immunized, challenged. | 4.15±0.19$^{ab}$ | 16.32±0.37$^{a}$ | 12.62±0.32$^{ab}$ | 38.47±0.43$^{bc}$ | 92.99±3.27$^{a}$ | 30.46±0.62$^{a}$ | 32.79±0.49$^{abc}$ |
| Group (10): 1000-*E. tsunodai* oocysts immunized, challenged. | 3.97±0.20$^{ab}$ | 16.48±0.38$^{a}$ | 12.35±0.39$^{bc}$ | 38.07±0.52$^{bc}$ | 96.34±3.71$^{a}$ | 31.19±0.61$^{a}$ | 32.43±0.63$^{abc}$ |

[1] Means: Mean of 3 replicates / group (Mean ± standard error).

[a-d] Means in the same column with different superscripts are significantly different at $P \leq 0.05$.

**Table 5:** Means of differential leukocyte count, 7 days post-challenge [1].

| Treatments | Differential leukocyte count (%) | | | | |
|---|---|---|---|---|---|
| | Lymphocytes | Heterophils | Basophils | Eosinophils | Monocytes |
| Group (1): Negative control. | 62.33±0.67$^{b}$ | 27.00±1.00$^{b}$ | 3.67±0.33$^{a}$ | 2.67±0.33$^{a}$ | 4.33±0.33$^{a}$ |
| Group (2): *E. bateri* positive control. | 62.00±1.15$^{b}$ | 34.33±0.88$^{a}$ | 1.33±0.33$^{b}$ | 0.67±0.33$^{b}$ | 1.67±0.33$^{bc}$ |
| Group (3): 100-*E. bateri* oocysts immunized, challenged. | 72.33±1.20$^{a}$ | 25.00±1.00$^{bcd}$ | 1.00±0.00$^{b}$ | 0.67±0.33$^{b}$ | 1.00±0.00$^{c}$ |
| Group (4): 1000-*E. bateri* oocysts immunized, challenged. | 74.00±0.58$^{a}$ | 23.33±0.88$^{cd}$ | 1.00±0.00$^{b}$ | 0.33±0.33$^{b}$ | 1.33±0.33$^{bc}$ |
| Group (5): *E. uzura* positive control. | 62.67±0.88$^{b}$ | 33.33±1.20$^{a}$ | 1.33±0.33$^{b}$ | 0.67±0.33$^{b}$ | 2.00±0.58$^{bc}$ |
| Group (6): 100-*E. uzura* oocysts immunized, challenged. | 71.67±0.88$^{a}$ | 24.67±1.20$^{bcd}$ | 1.00±0.00$^{b}$ | 1.00±0.00$^{b}$ | 1.67±0.33$^{bc}$ |
| Group (7): 1000-*E. uzura* oocysts immunized, challenged. | 74.00±0.58$^{a}$ | 22.33±0.88$^{d}$ | 1.33±0.33$^{b}$ | 0.67±0.33$^{b}$ | 1.67±0.33$^{bc}$ |
| Group (8): *E. tsunodai* positive control. | 63.33±0.88$^{b}$ | 33.67±0.88$^{a}$ | 1.00±0.00$^{b}$ | 0.67±0.33$^{b}$ | 1.33±0.33$^{bc}$ |
| Group (9): 100-*E. tsunodai* oocysts immunized, challenged. | 71.33±1.76$^{a}$ | 25.67±1.20$^{bc}$ | 1.00±0.00$^{b}$ | 0.67±0.33$^{b}$ | 1.33±0.33$^{bc}$ |
| Group (10): 1000-*E. tsunodai* oocysts immunized, challenged. | 72.33±1.20$^{a}$ | 23.33±0.88$^{cd}$ | 1.33±0.33$^{b}$ | 0.67±0.33 $^{b}$ | 2.33±0.33$^{b}$ |

[1] Means: Mean of 3 replicates / group (Mean ± standard error).

[a-d] Means in the same column with different superscripts are significantly different at $P \leq 0.05$.

oocysts immunized group had a significantly higher (P<0.05) weight gain than that of the 1000-*E. bateri* oocysts immunized group. Both *E. uzura* and *E. tsunodai* immunized groups possessed a significantly higher (P<0.001) weight gain compared to their positive controls. The groups immunized by 100-oocysts of *E. uzura* or *E. tsunodai* showed an insignificant improvement in the weight gain compared to the 1000-oocysts immunized groups (Table 3).

Regarding FCR, both 100 and 1000-*E. bateri* oocysts immunized groups showed a significant improvement (P<0.001) in FCR compared to the positive control group. The 100-*E. bateri* oocysts immunized group had a significantly lower FCR than that of the 1000-*E. bateri* oocysts immunized group. Both *E. uzura* and *E. tsunodai* immunized groups showed a significant improvement (P<0.001) in FCR compared to their positive controls. The groups immunized by 100-oocysts of *E. uzura* or *E. tsunodai* showed an insignificant lower FCR than those of 1000-oocysts immunized groups (Table 3) (Fig 10).

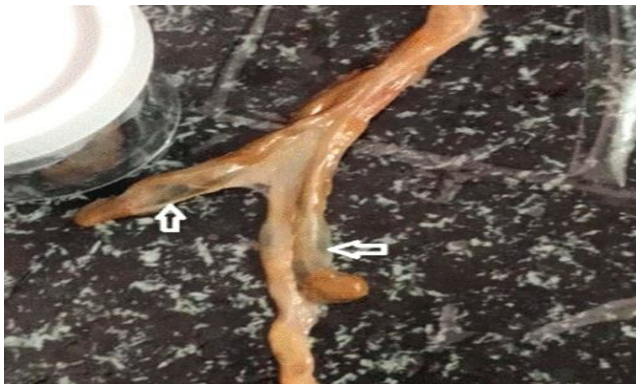

**Fig 9:** Ceca of a Japanese quail immunized by *E. tsunodai* 100-live sporulated oocysts and then challenged by $4\times10^4$ *E. tsunodai* oocysts, 4 weeks later, showed mild ballooning (arrows) with normal contents (score +1) (7th day post-challenge).

The improvement in the performance parameters caused by immunization may be attributed to the produced immunity resulting in suppression of parasite development and so lowering the intestinal lesions and disorders.

Concerning the hematological parameters, PCV values were significantly higher in the immunized groups of each *Eimeria* species compared to their positive controls except in case of the 1000-*E. bateri* oocysts immunized group where the increase was insignificant. There was an increase in Hb and WBCs values in the immunized groups compared to the positive controls. The positive control groups revealed a significant reduction in PCV and Hb values in comparison with the negative control group. MCHC value was significantly reduced in the *E. uzura* positive control group compared to the negative control group. RBCs, PCV, Hb and MCHC values were significantly reduced in *E. tsunodai* positive control group compared to the negative control group (Table 4) and this may be attributed to the hemorrhage and blood loose caused by this species. These negative impacts were reduced by immunization by *E. tsunodai* live oocysts, 100 and 1000-oocysts doses. In addition, the negative effects of coccidiosis on blood parameters may be due to the destructive effects caused by the endogenous developmental stages of *Eimeria* resulting in disturbances in the absorption of the essential nutrients and traces required for erythropoiesis and Hb synthesis as supported by Teixeira *et al.* (2004).

The differential leukocyte count revealed a significant increase in the number of lymphocytes in the immunized groups of each *Eimeria* species compared to the negative and positive controls. Scattered immunocytes (reactive lymphocytes) were found in the blood films of the immunized, challenged groups of each *Eimeria* species. Contrary, there was a significant increase in the number of heterophils in the positive controls compared to the immunized groups and the negative control group (Table 5). These observations can be explained by Campbell (1994) who mentioned that

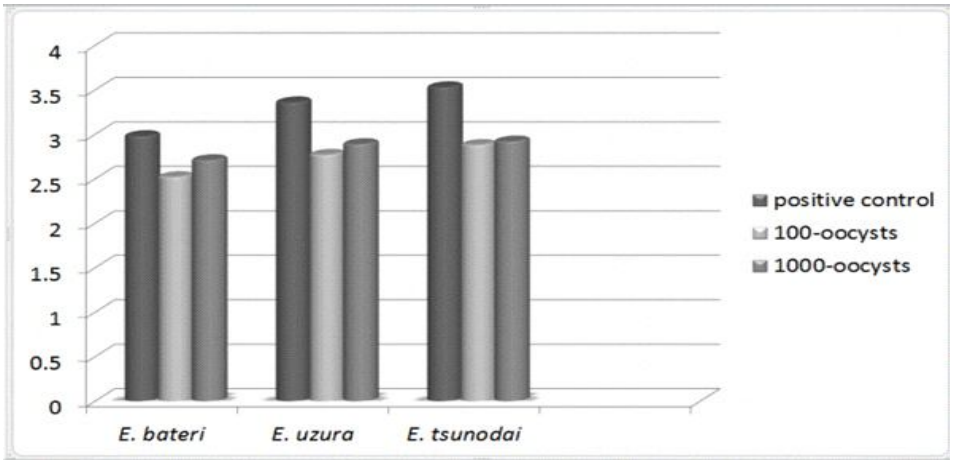


**Fig 10:** Means of FCR in different treatments of Japanese quails experimentally challenged with 3 *Eimeria* species separately.

scattered reactive lymphocytes may be noticed in the peripheral blood smear after antigenic stimulation by an immunization or a disease and that the infectious agents like bacteria, fungi, or parasites are associated with leukocytosis and heterophilia. The degree of heterophilia usually indicates the severity of the initial inflammatory process.

## CONCLUSION

We found that the immunization of Japanese quail by low doses of live sporulated oocysts of 3 *Eimeria* species separately gave excellent results, regarding all tested parameters, against the challenge by high doses. The 100-oocysts doses showed better results than the 1000-oocysts doses with insignificant differences. Therefore, further studies on coccidiosis vaccination in Japanese quail are needed such as the use of attenuated oocysts in vaccination, trials on different doses of live oocysts and vaccination of Japanese quails when reared in different systems.

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