

Pathomorphological changes in the alimentary system of Japanese quails naturally infected with *Eimeria tsunodai*

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Abstract

The aim of this study was to investigate pathomorphological changes in the duodenum, jejunum, ileum, and caecum of Japanese quails naturally infected with *Eimeria tsunodai*. Samples of the intestines were collected from 6-week-old cockerels and hens before laying and from laying quails aged 12, 24, and 48 weeks. The tissue sections were stained with haematoxylin and eosin, PAS method according to McManus, and Ziehl-Neelsen method. Morphometric and morphological analyses of coccidia revealed that *Eimeria tsunodai* was responsible for the infection. Different developmental stages of the coccidia were observed almost exclusively in the caecum, and they led to the complete damage of caecal mucosa. The main changes observed in the caecum involved mucosal damage, atrophy of the folds and crypts, and mucosal desquamation. No significant inflammation changes were detected. The degree of damage to caecal mucosa increased with age and the greatest damage was noted at the age of 48 weeks. *Eimeria tsunodai* infections occur in Japanese quails during the entire laying period. Different developmental stages of coccidia are responsible for total damage to caecal mucosa.

Key words: Japanese quails, *Eimeria tsunodai*, intestines, pathomorphology.

Introduction

Increasing consumers' awareness about the health benefits of food products has contributed to the popularity of quail eggs and Japanese quail farms in Poland. Modern quail production focuses on laying hens, whereas fewer farms breed Japanese quails for meat. Increased mortality of laying Japanese quails is caused by a growing number of pathogens, including coccidia, the predominant protozoal parasites in highly specialised production. Coccidiosis is a significant problem in quail farms due to the ban on feed supplementation with coccidiostats. The authors' experience indicates that coccidia, in particular parasites infecting Japanese quails, are highly resistant to popular antiprotozoal agents (unpublished data). Coccidiosis is very often a hidden disease, and necrotic enteritis or colibacillosis develop as a secondary infection. The presence of non-specific

clinical symptoms or unidentified mild clinical symptoms prevents from correct diagnosis. In such cases, the progression of coccidiosis may adopt a subclinical, chronic form (7). Three *Eimeria* species have been identified and described in Japanese quails (*Coturnix coturnix japonica*): *Eimeria uzura*, *E. bateri*, and *E. tsunodai* (2, 7, 8, 10). Depending on *Eimeria* species, parasites damage the mucosa of different parts of alimentary tract due to replication through a series of asexual and sexual cycles. There is no available literature describing pathomorphological changes in the alimentary system during coccidiosis in different age groups.

The aim of this study was to investigate pathomorphological changes in the duodenum, jejunum, ileum, and caecum of Japanese quails naturally infected with coccidia.

Material and Methods

The study of 45 Japanese quails (*Coturnix coturnix japonica*) was approved by the Local Ethics Committee. Birds were obtained from a commercial quail flock (10 000 birds in the farm). Quails were kept on litter until the 21st d of life, after which they were moved to cages. The quantitative composition of the feed till 21st d of life amounted 27% of total protein and 3000 kcal/kg metabolisable energy (ME), from 21st d to 42nd d 24% and 2900 kcal/kg, and from 42nd d 20% and 2800 kcal/kg, respectively. There was no vaccination programme in the flock.

Samples of the duodenum, jejunum, ileum, and caecum were collected from 6-week-old cockerels and hens before laying, and from laying quails aged 12, 24, and 48 weeks. Nine quails from different age groups were sacrificed. The samples were fixed in 10% neutralised formalin and embedded in paraffin blocks. Paraffin sections (5 µm) were stained with haematoxylin and eosin (HE), PAS method according to McManus, and Ziehl-Neelsen method (Ziehl-Neelsen Cryptosporidium kit, Bio-Optica, Italy), which confirms the detection of coccidia oocysts in tissue samples.

Two pathologists rated the samples without any knowledge of the age of quails. The degree of damage to the caecal mucosa was evaluated on a six-point scale, established by authors (Fig. 7): 0° - absence of morphological lesions with coccidia present; 1° - mild mucosal damage with insignificant shortening of the folds; 2° - moderate mucosal damage with shortening of the folds; 3° - prominent shortening of the folds with lumen extension; 4° - vestigial folds, atrophy of crypts; 5° - total atrophy of the mucosa (crypts and folds) with parchment-like membrane.

The images of tissue sections were acquired with the use of the MIDI 3DHISTECH Panoramic Scanner (Hungary). The images and measurement data were processed in Panoramic Viewer software (3DHISTECH, Hungary).

The Kolmogorov–Smirnov test with Lilliefors modification was performed to set compatibility of distribution of the samples. The Kruskal–Wallis non-parametric ANOVA test was performed to compare the means. Differences between age groups were statistically significant when the P-value for multiple comparisons was <0.05.

Results

Quail mortality increased in week three, and elevated mortality rates were noted throughout the laying period. Daily mortality rates reached 0.20%–0.23%, and they exceeded the norm of 0.08% to 0.1% in the production of laying quails. Necrotic enteritis was observed at different rearing stages. Body weight loss and dehydration were noted before death. Dead

birds were dehydrated and showed signs of muscular atrophy.

Sites of infection. Microscopic evaluations revealed pathomorphological changes in the alimentary tract, which were induced at different developmental stages of coccidia. The caecum was the most affected organ. The presence of parasites was observed in all examined caeca (Table 1). Coccidia in various developmental stages were noted in the caecal lumen and mucosa: merozoites (Figs 1–3, 5), meronts (Fig. 4), macrogametes (Fig. 3), microgametes, sporulated, and unsporulated oocysts (Figs 3, 6). Meronts were infrequently observed in the mucosa of the remaining parts of the intestinal tract. In the duodenum, single meronts were detected in one cockerel aged 6 weeks, in one hen of 12th and 24th week of life, and in four birds aged 48 weeks. Single meronts were reported in the jejunum of two cockerels and six hens aged 48 weeks. Ileal meronts were noted in only one cockerel aged six weeks, and in one hen in week 48. In the small intestine, coccidia were located in the upper section of the villi.

Description of coccidia. The developmental stages of coccidia were characterised based on the results of morphometric analyses. Oocysts in the caecal lumen had average dimensions of 18.34×14.71 µm. In the caecal mucosa, meronts were characterised by average dimensions of 11.56×7.99 µm, macrogametes - 16.49×8.39 µm, and zygotes - 12.05×10.44 µm. PAS staining revealed the presence of polysaccharides in coccidia. A positive reaction was only observed in meronts and oocysts. The Ziehl–Neelsen method revealed the presence of polysaccharides in oocysts of the caecal mucosa (Fig. 6), and minimum 10 oocysts were identified in each section.

Histopathological examination. The presence of parasites in sections of the intestinal tract other than the caecum was not associated with morphological changes. Low parasite counts did not have a damaging effect on the small intestinal mucosa. Infiltrating lymphoid and myeloid cells, mucosal desquamation and mild congestion were observed in the small intestine. Inflammatory infiltrate was noted in the duodenum (Table 1).

The presence of coccidia in different stages of the life cycle was reported in all examined caeca, and damage to the lamina propria was observed in all birds (Table 1). Caecal mucosa was damaged by the proliferation of parasites, meront growth, and release of merozoites (Figs 1, 2, 4, 5). Villus and crypt epithelial cells were damaged by multiplying coccidia. In many cases, the rapid proliferation of parasites and the vast populations of merozoites, which expand to the caecal mucosa in the second generation, led to the complete damage of the caecal mucosa, atrophy of Lieberkuhn crypts and folds (Figs 1, 2, 5, 7). The correlations between bird age and the degree of damage of the caecal mucosa are shown in Table 2.

Table 1. Number of birds with pathomorphological changes in the alimentary tract in different age groups

Pathomorphological changes	Site of pathomorphological changes in different age groups																			
	Duodenum					Jejunum					Ileum					Caecum				
	6	6	12	24	48	6	6	12	24	48	6	6	12	24	48	6	6	12	24	48
	♂	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♀	♂	♀	♀	♀	♀
	weeks					weeks					weeks					weeks				
Mucosal congestion	0/9	2/9	1/9	1/9	0/9	1/9	0/9	3/9	1/9	3/9	1/9	1/9	0/9	1/9	1/9	1/9	3/9	1/9	0/9	1/9
Villous oedema	0/9	0/9	3/9	3/9	2/9	0/9	0/9	1/9	1/9	0/9	0/9	0/9	1/9	0/9	0/9	2/9	1/9	0/9	2/9	1/9
Lymphoid cell infiltration	3/9	7/9	7/9	3/9	6/9	3/9	1/9	2/9	0/9	1/9	3/9	0/9	2/9	1/9	0/9	1/9	3/9	2/9	1/9	1/9
Myeloid cell infiltration	0/9	3/9	2/9	2/9	3/9	0/9	2/9	2/9	0/9	1/9	1/9	0/9	1/9	0/9	0/9	1/9	2/9	1/9	0/9	0/9
Mucosal desquamation	3/9	1/9	1/9	2/9	1/9	4/9	1/9	1/9	0/9	1/9	1/9	0/9	0/9	0/9	1/9	5/9	3/9	1/9	0/9	2/9
Presence of developmental stages of coccidia	1/9	0/9	1/9	1/9	4/9	2/9	0/9	0/9	0/9	6/9	1/9	0/9	0/9	0/9	1/9	9/9	9/9	9/9	9/9	9/9
Damage to villi/mucosa	0/9	0/9	2/9	1/9	1/9	1/9	0/9	1/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	9/9	9/9	9/9	9/9	9/9
Villous atrophy (atrophy of caecal folds)	0/9	0/9	1/9	0/9	0/9	0/9	2/9	4/9	3/9	4/9	0/9	0/9	4/9	0/9	4/9	6/9	6/9	6/9	9/9	9/9
Villous fusion	0/9	3/9	1/9	0/9	0/9	1/9	1/9	0/9	0/9	1/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9
Crypt atrophy	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	2/9	4/9	6/9	7/9	9/9
Hyperplasia of epithelial cells and crypts	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	5/9	8/9	2/9	3/9	6/9
Crypt dilation	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	2/9	1/9	1/9	1/9	0/9
Hypertrophy of epithelial cells	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	1/9	0/9	0/9	3/9
Total	7 _a	16 _c	19 _{d, e}	13 _{d, e}	17	12	7 _c	14	5 _d	17	7 _b	1 _c	8	2 _{d, e}	7 _f	43 _{a, b}	50 _c	38	41 _d	50 _f

a – statistically significant differences; b – statistically significant differences; Kruskal-Wallis test: $H(3, N = 52) = 13.36010$ $P = 0.0039$

c – statistically significant differences; Kruskal-Wallis test: $H(3, N = 52) = 22.69622$ $p = 0.0000$

d – statistically significant differences; e – statistically significant differences; Kruskal-Wallis test: $H(3, N = 52) = 10.6016$ $P = 0.0141$

f – statistically significant differences; Kruskal-Wallis test: $H(3, N = 52) = 8.489579$ $P = 0.0369$

Table 2. Number of birds with different degree of damage of the caecal mucosa in different age groups

Degree of caecal damage	Bird age (weeks) n = 9				
	6 ♂	6 ♀	12 ♀	24 ♀	48 ♀
	weeks				
0°	1/9	0/9	0/9	0/9	0/9
1°	1/9	1/9	2/9	0/9	0/9
2°	0/9	1/9	0/9	0/9	0/9
3°	3/9	3/9	1/9	3/9	2/9
4°	2/9	2/9	2/9	1/9	2/9
5°	2/9	2/9	4/9	4/9	5/9

The Kruskal-Wallis non-parametric ANOVA test (Table 1) revealed statistically significant differences between the caecum and duodenum, and between the caecum and ileum in six-week-old cockerels. Significant differences between the analysed parts of the alimentary system were noted in six-week-old hens while no differences were reported in 12-week-old birds. At 24 weeks, differences were reported between the caecum and the small intestine, and between the caecum and the ileum. Differences between the caecum and the ileum were observed in 48-week-old quails.

Damage of the lamina propria led to enteritis, higher mortality, metabolic disorders, and an overall deterioration of animal welfare.

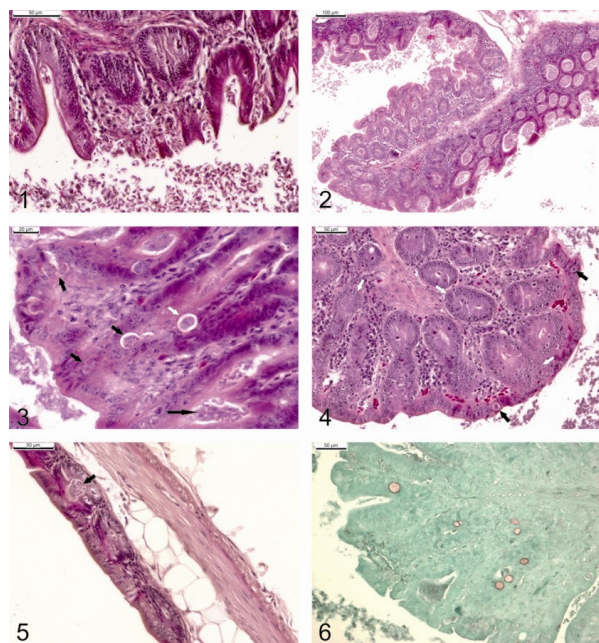


Fig. 1. Caecum - 24-week-old birds; Mucosa damaged by released merozoites. HE. About 200x

Fig. 2. Caecum - six-week-old birds; Large number of released merozoites, dilation of crypts. HE. About 40x

Fig. 3. Cecum - six-week-old birds; Mature macrogametes with wall-forming bodies (black arrow); merozoites in crypt lumina (long arrow); young oocysts with visible walls (white arrow). HE. About 240x

Fig. 4. Caecum - 12-week-old birds; developing meronts (black arrows); hyperplasia of epithelial cells and crypts (white arrows); visible mucosal congestion. HE. About 120x

Fig. 5. Caecum - 48-week-old birds; total damage and atrophy of the mucosa; meronts in crypts (arrows); parchment-like membrane. HE. About 240x

Fig. 6. Caecum - 12-week-old birds; young oocysts in the mucosa (red colour). Ziehl-Neelsen method. About 120x

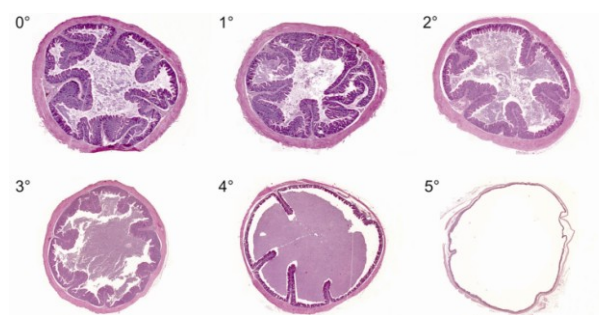


Fig. 7. Caecum. The degree of mucosa damage. 0° – absence of morphological lesions with coccidia present; 1° – mild mucosal damage with insignificant shortening of the folds; 2° – moderate mucosal damage with shortening of the folds; 3° – prominent shortening of the folds with lumen extension; 4° – vestigial folds, atrophy of crypts; 5° – total atrophy of the mucosa (crypts and folds) with parchment-like membrane. HE. About 4x

Discussion

Pathomorphological analysis of the alimentary tract in all bird groups revealed coccidia at various developmental stages almost exclusively in the caecum. The presence of single meronts was noted in the small intestine, without morphological changes.

Similar observations were made by Tsutsumi (10), who found *Eimeria tsunodai* only in the caecum. In our study, no considerable infection in the duodenum and jejunum was observed like in *Eimeria uruza* infection (6, 8), or in the duodenum and rectum like in *Eimeria bateri* infection (2).

The results of morphometric analyses of meronts, macrogametes, zygotes and oocysts were similar to the findings of Tsutsumi (10). Similarities were also reported in the PAS reaction. In our study, only second- and third- generation meronts and oocysts were PAS positive and similar results were reported by Tsutsumi (10), and Tsutsumi and Tsunoda (9). The analysed *Eimeria* species was identified as *Eimeria tsunodai*. This was based on the results of morphometric analyses, morphological characteristics, and location, which was almost exclusively restricted to the caecum.

The morphological changes observed in the caecum corresponded with the findings of Tsutsumi (10), and Tsutsumi and Tsunoda (9), who investigated *Eimeria tsunodai* infections. In the cited studies, epithelial cells were damaged with infiltration of eosinophils, lymphocytes, and plasma cells. Teixeira *et al.* (7) found that parasitic infections caused by *Eimeria tsunodai* led to villous erosion, hyperplasia of the crypts of Lieberkuhn, granulocyte and mononuclear cell infiltration, as well as oedema. Mohammad (3) studied infections caused by three *Eimeria* species and reported hyperplasia of epithelial cells, constriction of intestinal gland cavities in the small intestine and caecum, oedema between muscle fibers, and inflammatory cell infiltration. In our study, the presence of coccidia at various developmental stages in all bird groups was correlated with damage of epithelial cells in the folds and crypts and damage of the lamina propria. Interestingly, no significant immune response was noted in the damaged areas of the caecum. Eosinophil, heterophil, and mononuclear cell infiltrations were not observed. The presence of inflammatory cells in the caecal mucosa was unrelated to coccidiosis. Hyperplasia of epithelial cells was a sign of mucosal regeneration that often accompanies the states of physiological damage, including coccidiosis, which was also noted by Mohammad (3).

More profound changes were noted in older birds (48 weeks), which were characterised by a greater damage to the caecal mucosa than in younger birds. Our results are difficult to compare with the findings of other authors, who observed higher sensitivity to coccidiosis in younger birds (7-9 weeks of age) (2, 7). Our study focused on morphological characteristics without accounting for the infection rate. More advanced and health-threatening pathomorphological changes were noted in older quails. It should be noted, that the third, fourth, and fifth degree of caecal damage was more often diagnosed in birds at 48 weeks of age than in six-week-old birds.

In our study, quails were affected by a subclinical form of coccidiosis. Mortality rates were above the norm, non-specific symptoms were observed, and coccidiosis was the primary disease. A subclinical form of the disease was confirmed by Teixeira *et al.* (7). Mohammad (3) demonstrated that older quails developed immunity to the infection and showed non-specific symptoms characteristic of a mild immune response in the caecum.

The changes observed in the alimentary tract and the presence of coccidia in the entire examined bird groups raise questions about the legitimacy of the ban on feed supplementation with coccidiostats (4). National regulations clearly require the need for animal protection. In Poland, animal rights are addressed by the Animal Protection Act (1), which states that all animals should be humanely treated (Art. 5), and are entitled to legal protection in a manner appropriate to their needs (Art. 4). The welfare of domestic and farm animals should be protected through the provision of veterinary care and proper maintenance, which minimise pain and suffering (Art. 12).

Coccidiosis in Japanese quails remains a significant problem because coccidia are resistant to popular antiprotozoal agents (unpublished data). Chronic disease has adverse consequences for animal production, health, and welfare. There is a need for further discussion and advanced research on poultry feeding stuffs supplementation with coccidiostats (5).

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