

Micropathology of the Internal Organs of Japanese Quails Naturally Infected with *Eimeria tenella*

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ABSTRACT

Coccidiosis is a protozoan disease caused by *Eimeria bateri* (*E. bateri*), *Eimeria tsunodai* (*E. tsunodai*), *Eimeria uzura* (*E. uzura*), *Eimeria tenella* (*E. tenella*), *Eimeria necatrix* (*E. necatrix*), and *Eimeria acervulina* (*E. acervulina*). The goal of the current study was to explore the micropathology of the duodenum, jejunum, caecum, liver, lung, spleen, kidney, adrenal gland of Japanese quails naturally infected with *E. tenella*. The histopathological examination revealed that developmental *E. tenella* led to the damage of caecal, duodenal, and jejunal. Necrosis and desquamation of the integumentary epithelium, atrophy of crypts and folds, hemorrhages, lymphoid infiltration were confirmed in the mucous membrane of these intestines. The main changes observed in the parenchymal organs involved the fatty dystrophy of hepatocytes and lymphoid infiltration of parenchyma of the liver, stagnant hyperemia and edema of the lungs; granular dystrophy and necrosis of epithelial cells of the collecting ducts of the kidneys, venostasis of blood sinusoids of the spleen, hyperplasia of interrenal tissue, and dystrophia of suprarenal tissue of the adrenal gland. Morphometric studies have shown that pathological changes in the organs of quails infected with *E. tenella* led to a decrease in the thickness of the caecal mucosa, volume of the parabronchial lumen of the lung, and the number of renal corpuscles of the infected group, compared to the control group. The indicators of the interrenal-adrenal index of the adrenal glands, the number of clusters of lymphoid cells of the liver, and lymphoid nodules of the spleen increased. The received information could offer deep insights about pathogens in quails coccidiosis and can be used for planning therapeutic measures.

Keywords: *Eimeria tenella*, Internal organs, Japanese quail, Microscopic changes, Morphometrical indices

INTRODUCTION

Coccidiosis is a widely spread protozoan disease of birds caused by one-celled protozoa *Eimeria*, manifesting itself in acute and chronic forms (Shamim et al., 2015) and resulting in heavy economic losses of poultry farms (Vrba and Pakandl, 2014; Adhikari et al., 2020).

Coccidia is characterized by species specific to the host and location. This means that each species of coccidia parasitizes one host species or a few close host species. The usual localization of coccidia are intestinal cells, but a number of species also affect cells of other organs (Gajadhar et al., 2011; Berto et al., 2013). The most pathogenic species are *Eimeria maxima* (*E. maxima*), *Eimeria mitis* (*E. mitis*), *E. tenella*, *E. necatrix*, and *E. acervulina* for chickens (Sharma et al., 2015), *Eimeria anatis* (*E. anatis*), and *Eimeria butlaxhi* (*E. butlaxhi*) for ducks (Abdulla, 2010), *Eimeria anseris* (*E. anseris*), *Eimeria truncata* (*E. truncata*), and *Eimeria hermani* (*E.*

hermani) for geese (Song et al., 2017), *Eimeria dispersa* (*E. dispersa*), *Eimeria gallopavonis* (*E. gallopavonis*), *Eimeria meleagritidis* (*E. meleagritidis*), and *Eimeria innocua* (*E. innocua*) for turkeys (Vrba and Pakandl, 2014).

Quails are very sensitive to coccidiosis as confirmed by the results of both experimental and natural infections. The disease is often caused by some types of agents which parasitize together, such as *E. bateri*, *E. tsunodai*, *E. uzura*, *E. tenella*, *E. necatrix*, and *E. acervulina* (Umar et al., 2014; Arafat and Abbas, 2018; Kot et al., 2020). Quails monoinvasion with coccidia is seldom observed (Gesek et al., 2014).

As to the area of localization in the intestinal tract of poultry, *E. tenella* and *E. tsunodai* infect caecum (Patra et al., 2009; El-Morsy et al., 2016), moreover, *E. necatrix* and *E. anseris* infect jejunum and ileum (Song et al., 2017; Sawale et al., 2018). In wild birds, extraintestinal forms of

coccidiosis are caused by *Eimeria reichenowi* (*E. reichenowi*) (Bertam et al., 2015; Jankovsky et al., 2017).

Some postmortal and microscopic changes which are typical for catarrhal, catarrhal-haemorrhagic, haemorrhagic, fibrinonerotic black scour, and haemorrhagic typhlitis are reported for the intestinal coccidiosis of birds (Song et al., 2017; Sawale et al., 2018; Kumar et al., 2019).

The results of the postmortal examination of the parenchymal organs of coccidiosis in birds are insufficiently described in the related literature and they are preferably concerned with the extraintestinal form of a given disease (Novilla et al., 1989; Morgan et al., 2013; Jankovsky et al., 2017).

The aim of this work was to study the morphological changes in the microscopic structures of the duodenum, jejunum, caecum end, liver, lung, spleen, kidney, adrenal gland of Japanese quails under natural *E. tenella* invasion that will broaden the knowledge about the pathogenesis of coccidiosis in given species of birds.

MATERIALS AND METHODS

Ethical approval

All animal experiments were conducted in accordance with the Law of Ukraine “On the Protection of Animals from Brutal Treatment” and the recommendations of the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Animals and study design

Clinically healthy (12 Japanese quails) and infected male (12 Japanese quails) with coccidiosis aged 45 days old were isolated for the study from agricultural holding “Mykolai” (Zhytomyr Oblast, Ukraine), then moved to the research-clinic diagnostic laboratory of the Faculty of Veterinary Medicine (Polissia National University, Zhytomyr, Ukraine) for conducting a histological and comparative morphometrical study of organs. Accompanying documents informed that according to the results of epizootological and parasitological research conducted in Zhytomyr state laboratory (Food Quality and Consumers Protection Service of Ukraine), sick quails were infected naturally with *E. tenella* (invasion intensity equaled 5280 oocysts per 1g of poultry litter).

Histological examination of quails organs was preceded by an anatomic study that included poultry harvesting and bleeding, autopsy of chest and belly cavity,

and organs section with their further removal from the cavity.

Poultry harvesting took place after inhalation of chloroform overdose using an acute bleeding technique by cutting the subclavian artery (Brooks Brownlie and Munro, 2016). The anatomic section of the duodenum, jejunum, caecum, liver, lung, spleen, kidney, adrenal gland of quails was performed after the autopsy of the chest and belly cavity (Reavill and Schmidt, 2019). Tissue samples (2cm) were taken from the mentioned organs, fixated in 10% water neutral solution of formaldehyde, dehydrated in ethyl alcohol with increasing concentrations to 40%, 70%, 96%, and 100%, inspissated in spiritus-dimethylbenzene (1:1) and two portions of dimethyl benzene, and doused in wax under the temperature of 60°C. Slices (5-8 mkm) were cut from wax blocks on the sliding microtome MC-2, put on the watch glasses, and stained with hematoxylin and eosin (Mulisch and Welsch, 2015).

The analysis and microphotography of histological preparations were conducted with a digital camera, mounted into the microscope Primo Star (Carl Zeiss, Germany), and connected to a personal computer.

Morphometrical techniques were used to get objective comparative data of the structural organization of the tested organs of clinically healthy and sick quails. The WCIF ImageJ (WCIF, Canada, 2000) software was used in this regard. The results included the indices of the thickness of the mucous lining of caecum ends and volume of parabronchi lumens, and interrenal-suprarenal index of an adrenal gland, as well as the number of renal corpuscles, lymphoid cells of the liver, and lymph nodules of a spleen per 100 mkm².

Statistical analysis

Digital data of morphometrical research was processed by applying variation-statistical methods using a software package “Statistica 6” (Stat Soft Inc., USA). The analysis of the received data was based on the indicators of descriptive statistics (including arithmetic mean, average mean inaccuracy). The reliability of the received data was estimated by Fisher F-criteria. The difference between the two values was considered significant when $p < 0.05$.

RESULTS

The histological examination of quails caecum infected with coccidia *E. tenella* indicated the destruction of a mucous lining up to crypts level (Figure 1A). According to

morphometrical examination, the thickness of the mucous lining of the caecum was equal to 93.16 ± 12.47 mkm, which is by a factor of 4.57 less than the same index of healthy animals reported as 425.83 ± 36.04 mkm ($p < 0.05$).

The layer of the mucous lining of the caecum ends was swollen and engorged with blood. The cells of the basal membrane and the crypts epithelium were in a chaotic state and necrotized. The crypts' boundaries and their lumens were not visualized. In some places, the remnants of the structural elements of mucous lining were covered with a layer of a conglomerate of a necrotic detritus, forming blood elements and oocysts (Sporozoites, Figure 1B). The content of an analogical composition was observed in the caecum ends lumen. The mucous lining of the caecum ends was swollen, its cells were in a state of albuminous swelling.

Some microscopic changes in the villi of a mucous lining were found in the duodenum and jejunum of sick quails. The villi had different heights and lost their characteristic form because of the destruction of their apical parts. They were tangent and overlapped each other forming a shapeless mass (Figure 2A). The cells of the germinal epithelium of tested bowels had eosinophilic cytoplasm, some nuclei were hyperchromatic, and others were in a state of karyolysis or karyorrhexis. In some places on the lateral borders of the villi matrix of a mucous lining, the germinal epithelium was desquamated. Its segments, together with crypt cell production, white blood cells, and solitary eimeria (meront) or their groups, tightly filled the bowel's lumen. The layers of submucous and the mucous lining of tested bowels swelled with hemorrhages (Figure 2B). An inflammable infiltrate was observed between intestinal glands. At the bottom of the crypt, the epithelial layer remained which was presented by goblet-shaped cells in a state of hypersecretion. A broadened apical part of these cells contained secretory granules, and a constricted part (basal) contained a nucleus.

The histological examination indicated a chaotic state of hepatic plates in quails infected with coccidia *E. tenella*. Most of the hepatocytes showed evidence of fatty dystrophy. Considering the infiltration type of the given pathology, the hepatocytes took a ring-like shape, drops of fat were registered in the cytoplasm indicating the presence of a nucleus in the periphery position. Regarding fatty dystrophy, the hepatocytes were of a round shape by the type of atrepsy showing the nucleus in the center (Figure 3A). In some hepatocytes the cytoplasm was nonhomogenous, of granular and foamy look, the nuclei were with the signs of lysis and pyknosis (granular

dystrophy).

The micropathology of the structural elements of hepatic triads was manifested by broadening of the vein lumen, through their filling with thickened plasma and gluten blood corpuscles. The lumen of interlobular arteries, on the contrary, was narrowed. Some lymphoid clumps, which consisted mainly of little and medium lymphocytes, were observed nearby (Figure 3A).

Hemo-sinusoidal capillaries could be seen among the hepatic plates which were in a chaotic state. Their lumen was dilatated, it sometimes contained red blood cells. On some areas of the liver tissue specimen, by the direction of hemo sinusoidal capillaries, there was a diffuse clump of the cells of a lymphoid group (Figure 3B). According to a morphometrical study of sick quails liver, the number of clumps of the cells of the lymphoid group per relative unit of liver area was equal to 3.19 ± 0.21 units, it is by a factor of 2.61 larger ($p < 0.05$), compared with the same indicator in quails of a control group estimated as 1.22 ± 0.15 units.

The proliferation of endotheliocytes and deep cells in the renal capsules of intercurrent and cortical nephrons, the ectasia of blood capillaries, and as a result, the increase in the size of a vascular sling of hemo-capillaries have been detected by the histological examination of kidney in sick quails under coccidiosis. The widening of capsule teeth associated with the deformation of renal corpuscles could be observed in some places. The results of the morphometrical study showed that the number of renal corpuscles per relative unit of kidney area of sick quails (18.05 ± 1.57) was significantly less by a factor of 1.45 ($p < 0.05$) than the same indicator of intact quails (12.46 ± 1.09).

In proximal convoluted and straight tubules of a nephron, the boundaries between the epithelial cells were non-visualized. Their nuclei could not be stained, the cytoplasm was in some degree exposed to lysis, preferably in a basal part. In collecting ducts the epithelial cells were in a chaotic state, increased in volume, their cytoplasm contained granules of protein nature. In some places, epitheliocytes protruded into the lumens of collecting ducts which were partly filled with a homogenous or closed-grained mass of protein. Regarding epithelial dystrophy and necrosis, its desquamation in the lumens of bellini ducts was observed (Figure 4A). The kidney stroma was swollen, there were hemorrhages between the capsular teeth and the ducts. The venules and the veins were distended and engorged with blood cells (Figure 4B). The destruction of the wall of central veins resulted in blood penetrating the Billini ducts lumens.

Regarding quails coccidiosis caused by coccidia *E. tenella*, lung particles were surrounded by the layers of loose fibrous connective tissue in which the vessels overflowed with blood presented. Lung parenchyma was in some places infiltrated with the cells of a lymphoid group, the vessels were congested with blood, and the connective tissue got swollen under vessels permeability. Parabronchi atria were widened, of an oval form, congested with blood. The parabronchi lumen was narrowed, contained transudate, lymphocytes, red blood cells, and desquamated epitheliocytes (Figure 5A). The results of the morphometrical examination showed that the volume of parabronchi lumen in sick quails under coccidiosis was equal to 20.27 ± 1.46 thous. mkm^2 , it is by a factor of 1.33 less ($p < 0.05$) than the same index in quails of a control group (26.89 ± 1.46 thous. mkm^2).

The induration of the wall of the arteria vessels, due to arteria vessels swelling, could be observed on some areas of tissue specimen of sick quails lung. This swelling reached perivascular parts of lung tissue. The volume of fibrous structures in the arteries wall increased, and tumor fluid accumulation could be found around the fibrous structures. All the arteries' lumen was full of red blood cells and desquamated endothelium (Figure 5B). The lumens of separate pneumo-capillaries were narrowed, and full of desquamated respiratory epithelium.

In quails infected with coccidia *E. tenella*, red and white parenchyma pulp were not differentiated. Venous sinusoids of red pulp were distended and congested with blood (Figure 6A). The findings indicated the intensive development of white pulp in close connection with the walls of arteria and arterioles in the form of lymph nodules

with germinal centers. Lymph nodules were of a round or oval form and were located in different parts of the spleen parenchyma. According to morphometrical results of the study, the number of lymph nodules per relative unit of quails spleen area under coccidiosis was equal to 4.27 ± 0.23 , it is by a factor of 1.96 more ($p < 0.05$) than the same index in the quails of a control group (2.18 ± 0.16). Germinal centers occupied the central part of lymph nodules and contained light centers (secondary lymph nodules). Periarterial lymphocyte sheaths were of different forms (round, oval, granular). Sinusoidal hemo-capillaries were distended and full of red blood cells (Figure 6B).

The histologic examination of the quails' adrenal gland infected with coccidiosis showed that cell bundles of interrenal tissue were in a chaotic state, and the endocrine cells were located in disorder (Figure 7A). Nuclei basophilia, hypertrophy of cytoplasm were observed and cells polymorphism and two-nuclei cells were found in some places. According to the morphometrical findings, in quails infected with coccidia *E. tenella*, the interrenal-suprarenal index ($1.94 \pm 0.03\%$) exceeded the same index in quails of a control group ($1.22 \pm 0.02\%$) by a factor of 1.59 ($p < 0.05$).

The lumens of sinusoidal hemo-capillaries and that of venous sinuses of an adrenal gland of sick quails were widened and congested. In some places, the exit of red blood cells out of sinusoidal hemo-capillaries into perivascular areas in a form of the focal collection was observed, Endocrine-cells of suprarenal tissue had signs of karyopyknosis, plasmaticpynosis, and plasmolysis (Figure 7B).

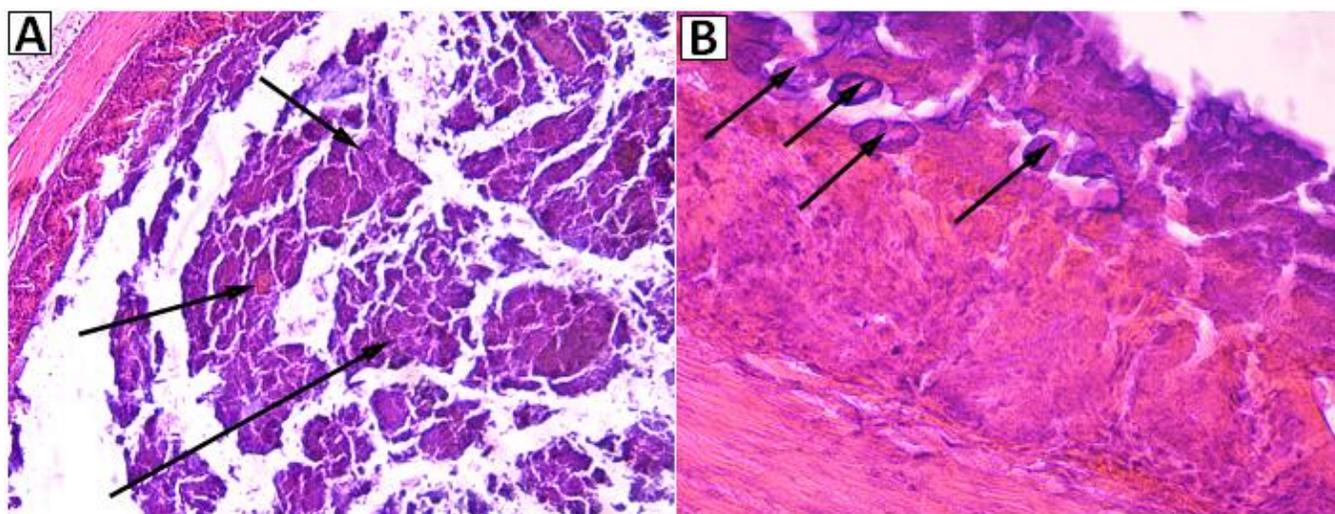


Figure 1. The histopathological picture of some changes in the caecum of 45-day-old Japanese quails under coccidiosis. **A:** The desquamation of the structural elements of a mucous lining in a lumen of the caecum, **B:** Coccidia oocysts on the area of necrosed crypts of a mucous lining, H&E $\times 400$

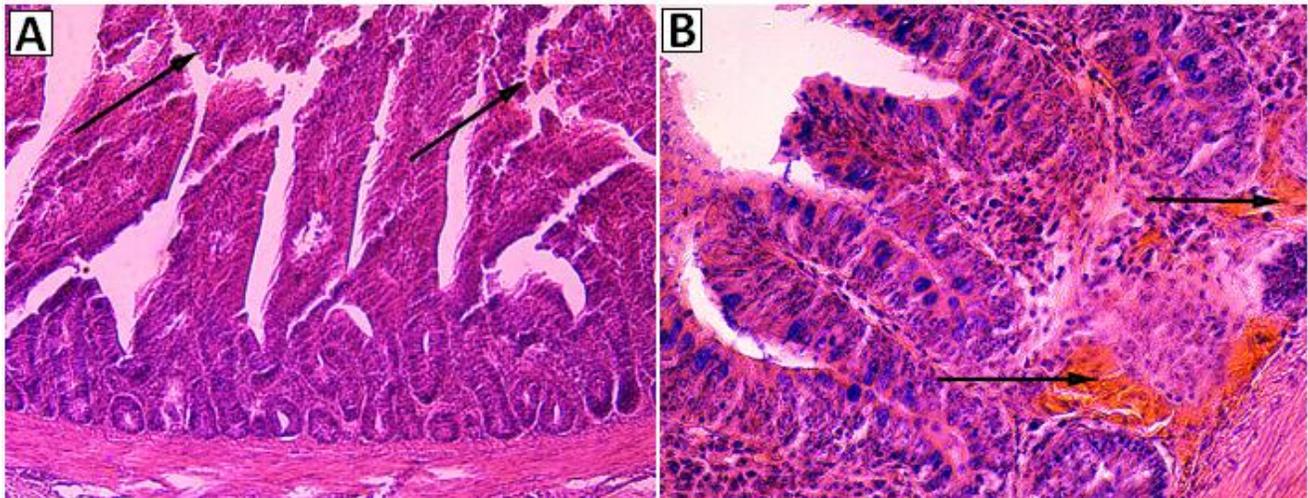


Figure 2. The histopathological changes in the duodenum (**A**) and in jejunum (**B**) bowels of a 45-day-old Japanese quail under coccidiosis. **A:** The destruction of the epithelial villi corpuscles of a mucous lining, **B:** Swelling and hemorrhages in the submucous matrix and a mucous lining plate, H&E \times 400

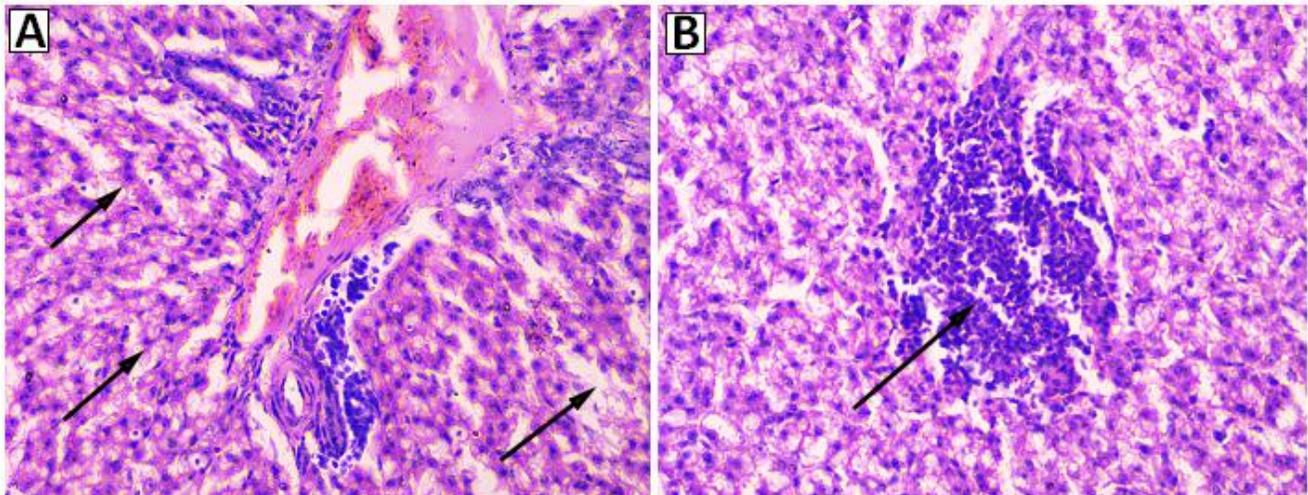


Figure 3. Histopathological changes in the liver of 45-day old Japanese quails under coccidiosis. **A:** Fatty dystrophy of hepatocytes, **B:** Lymphoid infiltration, H&E \times 400

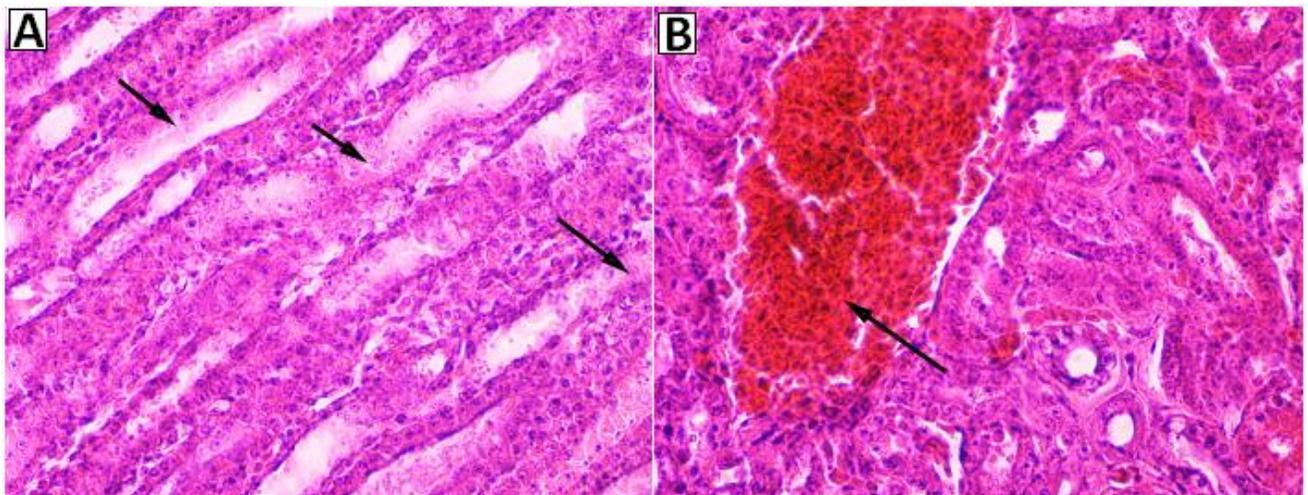


Figure 4. Histopathological changes in kidney of 45-day old Japanese quails under coccidiosis. **A:** Necrosis and desquamation of epithelial collecting ducts, **B:** Hyperemia of parenchyma, hemorrhages, blood congestion in a central vein, H&E \times 400

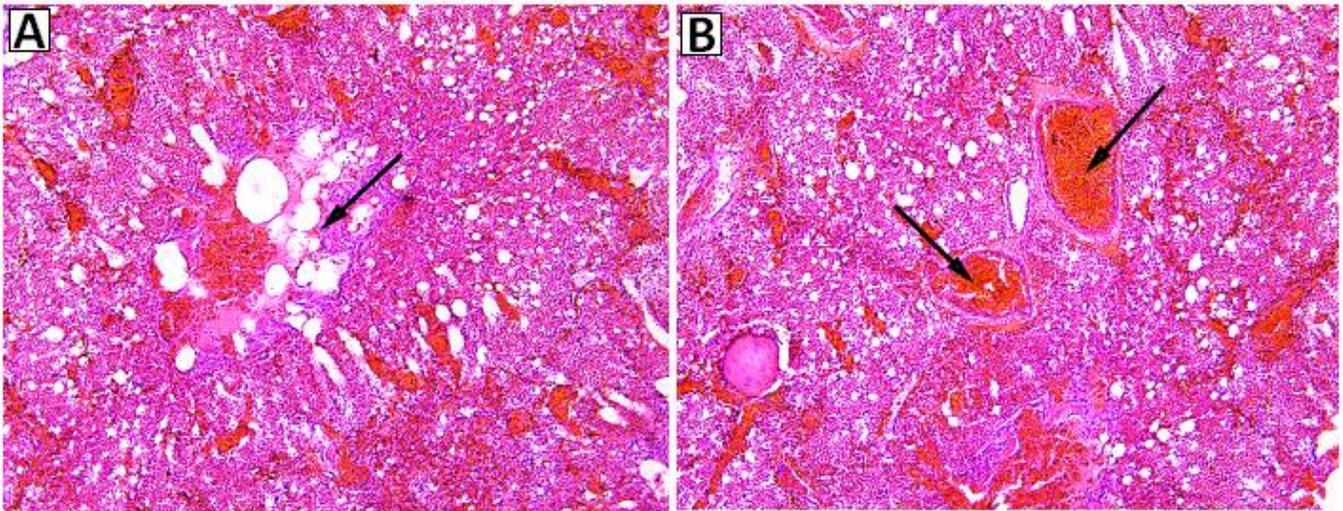


Figure 5. Histopathological changes in lung of 45-day old Japanese quails under coccidiosis. **A:** Desquamation of epitheliocytes into parabronchi lumen, **B:** Perivascular swelling, blood congestion in vessels, H&E $\times 100$

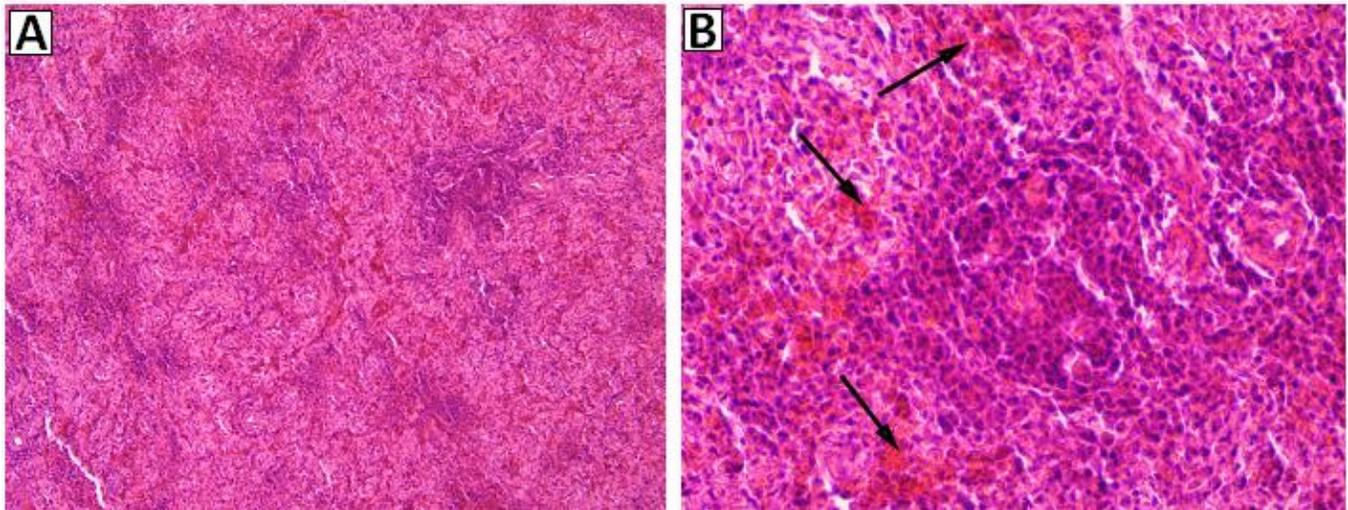


Figure 6. Histopathological changes in a spleen of 45-day old Japanese quails under coccidiosis. **A:** Significant congestion of red pulp with blood, **B:** Perivascular swellings, H&E $\times 400$

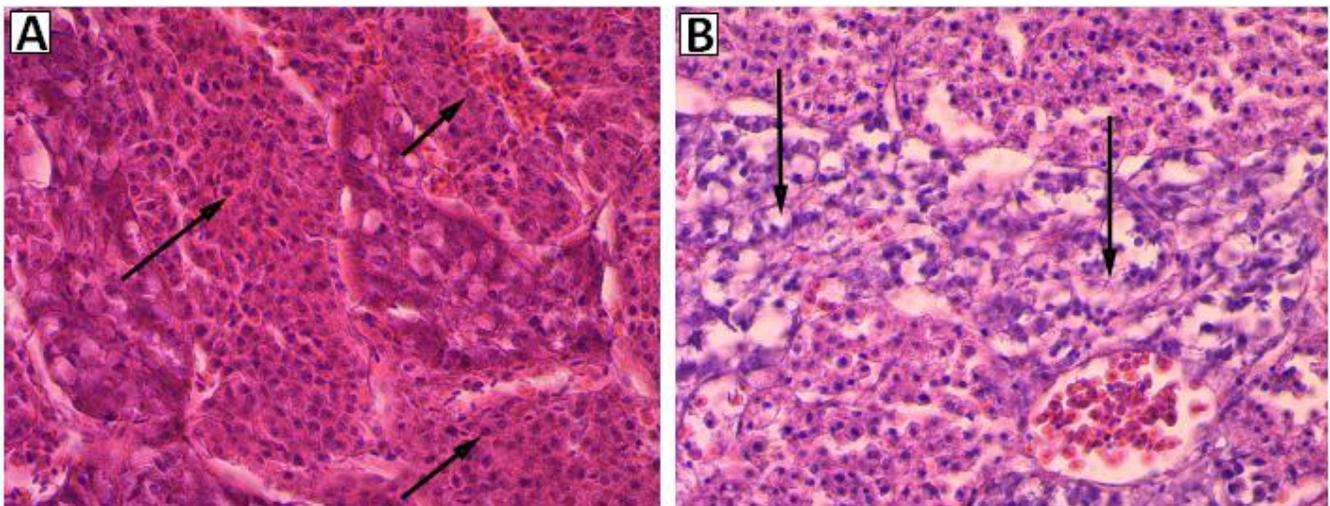


Figure 7. Histopathological changes in an adrenal gland of 45-day old Japanese quails infected with coccidiosis. **A:** Hyperplasia of an interrenal tissue, **B:** Dystrophia and necrobiosis of endocrine-cells of a suprarenal tissue, H&E $\times 400$

DISCUSSION

The obtained results of the current research indicated that microscopic changes in the internal organs of quails with coccidiosis induced by *E. tenella* invasion were systemic. Caecum of the quails' digestive system was most infected, as it was reported by Gesek et al. (2014), who studied the development of coccidia *E. tsunodai* in a mucous lining of caecum associated with necrosis and desquamation of its surface epithelium, crypts, and folds atrophy. In previous studies, some microscopic changes in catarrhal mucous, serous and hemorrhagic inflammation of caecum ends under natural quails invasion with coccidia of (*E. tenella*, *E. necatrix*, and *E. acervulina*) were noted (Kot et al., 2020). Mohammad (2012) and Gesek et al. (2014) pointed out the increase in the amount of immune-competent cells around the mucous lining crypts of quails caecum end infected with coccidiosis invasion (*E. tsunodai*, *E. uzura*, and *E. bateri*), as a result of protective and adaptive reactions under a local action of harmful products of coccidia vital activity.

According to the present morphometrical study, the mucous lining thickness of sick quails caecum with coccidiosis decreased by the factor of 4.57 ($p < 0.05$), compared with the same index of clinically healthy quails. This finding contradicts Mohammad's (2012) data on epitheliocytes hyperplasia associated with mucous lining thickness of the caecum ends of quails infected with *E. tsunodai*, *E. uzura*, *E. bateri*. According to Mohammad (2012), such changes could confirm the renewal of the structural elements of the mucous lining of caecum ends damaged by the disease.

The coccidia development in the mucous lining of quails caecum and the destruction of its cell elements resulted in the growth of pathogenic microflora which complicated the inflammatory processes in the small bowel and led to its dysperistalsis (Gajadhar et al., 2011; Song et al., 2017; Sawale et al., 2018). Under the histological examination of quails' duodenum and jejunum infected with natural invasion with *E. tenella*, there were microscopic signs of catarrhal-desquamated inflammation. It coincided with the findings of a study by Umar et al. (2014) as to the fact that in the quails infected with *E. bateri*, the apical part of the villi of the mucous lining of a duodenum had no surface epithelium, the desquamated epitheliocytes on different stages of destruction were surrounded by crypt-cell product and white blood cells, isolated sporozoids. Similar microscopic changes were noticed under the previous histological examination of a mucous lining of quails' duodenum and jejunum infected with coccidia of *E. tenella*, *E. necatrix*, *E. acervulina* (Kot

et al., 2020). According to Song et al. (2017), in geese that were experimentally infected with *E. anseris*, the coccidia development could be found in epitheliocytes cytoplasm of the crypts of a mucous lining of a jejunum as well as of ileum, which was associated with desquamation, necrosis of a surface and glandular epithelium of a mucous lining, as well as its swelling and infiltration with the cells of a lymph group.

Liver, as an organ which is on the way of blood current from the intestine to the blood path, is a barrier for toxic substances of endogenous and exogenous origin, including harmful substances of coccidia vital activity (Doneley, 2004; Gajadhar et al., 2011). Some microscopic changes which are typical for protein and fatty dystrophy were found by a histological examination of quails liver infected by *E. tenella*. The morphometrical study determined an increase in the amount of accumulation of cells of lymph group around the interlobular arteries and sinusoidal hemo-capillaries by a factor of 2.61 ($p < 0.05$), compared with clinically healthy quails, that confirmed immunity stress and the enhancement of a barrier function of an ectopic lymphoid tissue of a liver.

According to Ruff and Allen (1982), Patra et al. (2009), and Zaefarian et al. (2019), burned-out liver cells fail to synthesize glycogen, glucose, factors of prothrombin, and albumens, take part in aminoacids exchange as well as in fatty acids and other metabolic products exchange, utilize ammonia and other harmful products, conjugate bilirubin. The liver barrier function depression is followed by the accumulation of harmful substances in the blood and in tissues which cause dystrophy and necrosis in other organs.

Among urination organs in birds infected with coccidiosis, kidneys are the most affected organ (Bertam et al., 2015; Jankovsky et al., 2017). Present histological analysis of quails kidney infected with *E. tenella* showed some microscopic changes typical of proliferative intercapillary glomerulonephritis, granular dystrophy of collecting ducts epitheliocytes, and of venous hyperemia. Herewith, renal corpuscles and proximal tubules were most affected, the structural kidney elements which are characterized by a complexity of a structure and by an intensive course of energy processes. The reabsorption of proteins, glucose, electrolytes, water, and the excretion of by-products as well as many toxic substances from primary urine into blood happens in proximal convoluted and straight tubules of a nephron (Bertam et al., 2015; Jankovsky et al., 2017). In the current study, the toxic products of coccidia *E. tenella* vital activity were not excluded which caused significant while-alive distortions in a transepithelial movement of substances and resulted in

lysis of epitheliocytes cytoplasm of the renal tubule. It was established by the morphometric findings of the current research that the number of renal corpuscles per relative unit of kidney area of the infected quails reduced by a factor of 1.45 ($p < 0.05$) as compared with the same index for the quails in a control group, which was partly the result of proliferative inter-capillary glomerulonephritis found by a histological examination. A similar micropathology of the kidneys was detected by Jankovsky et al. (2017) and Morgan et al. (2013) when studying the pathogenesis of the renal form of extra-intestinal coccidiosis of the large-horned owl and apteryx. These authors also confirmed necrosis and obstruction of renal corpuscles, hyperplasia of bellini ducts. In the present research hyperemia and hemorrhages of the kidney, parenchyma was reported as it was recorded in the previous histological examinations of quails coccidiosis caused by *E. tenella*, *E. necatrix*, and *E. acervulina* (Kot et al., 2020).

The respiratory organs of birds infected with coccidiosis can also have pathological changes (Novilla et al., 1989; Morgan et al., 2013; Kot et al., 2020). According to the present investigation, natural *E. tenella* invasion causes local disturbed blood circulation (hyperemia, hemorrhages) and distortion of the processes of transudation (swelling of perivascular and peribronchial connective tissue) under the accumulation of transudate, lymphocytes, red blood cells, and desquamated epitheliocytes in the lumens of lung parabronchi. The results of the morphometrical study showed that the volume of parabronchi lumens of sick quails is by a factor of 1.33 ($p < 0.05$) less than the same index in clinically healthy quails, which could be indicative of external respiration problems. Analogical microscopic changes were reported in previous histological examinations of quails' lung, infected with *E. tenella*, *E. necatrix*, and *E. acervulina* (Kot et al., 2020). Microscopic signs of granulomatous lung fever were also observed under the histological examination of whooping crane lung under disseminated visceral coccidiosis caused by *E. reichenowi* and *E. gruis* Novilla et al. (1989). The granulomas and granulomatous contained a great number of meronts and mononuclear cells. Analogical data was recorded by Morgan et al. (2013), who studied the pathogenesis of a pulmonary form of extraintestinal coccidiosis in apteryx.

Domestic quails differ from other poultry in terms of intensive metabolism and higher body temperature, which make them resistant to many diseases (Seleznev et al., 2015; Soutter et al., 2020). According to the current histological examination of quails spleen infected with *E.*

tenella, there were instances of hyperemia of venous sinusoids of red pulp, as well as an intensive development of white pulp along with the wall of arteries and arterioles in the form of lymph nodules with germinal centers. The morphometrical examination indicated an increase in the number of lymph nodules per relative unit of spleen area of sick quails by a factor of 1.96, compared with the same index in clinically healthy quails of a control group. Such microscopic and morphometrical changes can be explained by a morphofunctional maturity of lymphoid tissue of a spleen of the infected quails as well as the improvement of an immune process, directed against the agent to eliminate its toxins. It contradicts with the results of the study by Kot et al. (2020) when under quails invasion with few species of coccidia (*E. tsunodai*, *E. uzura*, *E. bateri*) it is observed microscopic changes of perivascular and perinuclear swelling, depression of a lymphopoietic function of a spleen. Regarding the splenic form of extraintestinal coccidiosis in apteryx, Morgan et al. (2013) reported the accumulation of meronts in the parenchyma of an organ.

From among the peripheral organs of quails endocrine system, an adrenal gland is of great importance for the vital activity of an organism, as its hormones affect the resistance ability of an organism against infection, intoxication, and stress (Spencer et al., 2009; Scanes, 2016; Lotveld et al., 2017). According to the histological examination of quails' adrenal gland infected with *E. tenella*, some microscopic signs of hyperplasia of endocrine cells of an interrenal tissue were observed that caused the increase in the interrenal-suprarenal index by a factor of 1.59 ($p < 0.05$), compared with the same index of an adrenal gland of clinically healthy quails. Some microscopic signs of dystrophy and necrobiosis associated with swelling, distention, and blood filling of venous sinuses were indicative of a long stress-reaction of an adrenal gland of sick quails.

CONCLUSION

The microscopic changes in the duodenum, jejunum, caecum, liver, lung, spleen, kidney, adrenal gland of Japanese quails naturally infected with *E. tenella* were manifested in a form of inflammatory changes, hemodynamic abnormalities, and compensatory adaptive processes. These data conform to changes in the thickness of mucous lining of the caecum, as well as to the volume of the lumen of lung parabronchi, number of renal corpuscles, accumulation of lymphoid cells of liver, and lymphoid nodules of the spleen, and interrenal-suprarenal index of an adrenal gland. The obtained results could

deepen the insights about pathogenesis in quails coccidiosis leading to effective planning for therapeutic measures.

DECLARATIONS

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Authors' contribution

Oleksandr Rudik and Tetiana Kot created the idea and designed the research, Svitlana Gural'ska wrote a draft of the manuscript. Yuriy Dovhiy and Olena Zhytova collected data and performed the statistical analysis. All authors read and approved the final manuscript.

Competing interests

The authors have declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

REFERENCES

- Abdulla DA (2010). Coccidiosis in domesticated ducks in Ninevah governorate. *Iraqi Journal of Veterinary Science*, 24(2): 149-153. DOI: <https://www.doi.org/10.33899/ijvs.2010.5602>
- Adhikari P, Kiess A, Adhikari R, and Jha R (2020). An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. *Journal of Applied Poultry Research*, 29(2): 515-534. DOI: <https://www.doi.org/10.1016/j.japr.2019.11.005>
- Arafat N, and Abbas I (2018). Coccidia of Japanese quail: from identification, prevalence, infection and immunization. *The Journal of Parasitology*, 104(1): 23-30. DOI: <https://www.doi.org/10.1645/17-109>
- Bertam M, Hamer G, Snowden K, Hartup B and Hamer S (2015). Coccidian parasites and conservation implications for the endangered whooping crane (*Grus americana*). *Plos One*, 10(6): e0127679. DOI: <https://www.doi.org/10.1371/journal.pone.0127679>
- Berto BP, Borba HR, Lima VM, Flausino W, Teixeira-Filho WL, and Lopes CW (2013). *Eimeria* spp. from Japanese quails (*Coturnix japonica*): new characteristic features and diagnostic tools. *Pesquisa Veterinaria Brasileira*, 33(12): 1441-1447. DOI: <https://www.doi.org/10.1590/S0100-736X2013001200008>
- Brooks Brownlie H, and Munro R (2016). The veterinary forensic necropsy: A review of procedures and protocols. *Veterinary Pathology*, 53(5): 919-928. DOI: <https://www.doi.org/10.1177/0300985816655851>
- Doneley B (2004). Treating liver disease in the avian patient. *Seminar in Avian and Exotic Pet Medicine*, 13(1): 8-15. DOI: [https://www.doi.org/10.1053/S1055-937X\(03\)00053-7](https://www.doi.org/10.1053/S1055-937X(03)00053-7)
- El-Morsy MA, Abou El-Azm KI, and Awad SS (2016). Efficacy of Some Anticoccidial Drugs on Experimentally Induced Cecal Coccidiosis (*E. tsunodai*) in Japanese Quails. *The Egyptian Journal of Veterinary Science*, 47(2): 165-177. DOI: <https://www.doi.org/10.21608/EJVS.2017.3591>
- Gajadhar A, Wobeser G, and Stockdale P (2011). Coccidia of domestic and wild waterfowl (Anseriformes). *Canadian Journal of Zoology*, 61(1): 1-24. DOI: <https://www.doi.org/10.1139/z83-001>
- Gesek M, Welenc J, Tylicka Z, Otrocka-Domagala I, Pczdzior K, and Rotkiewicz A (2014). Pathomorphological changes in the alimentary system of Japanese quails naturally infected with *Eimeria tsunodai*. *Bulletin of the Veterinary Institute in Pulawy*, 58(1): 41-45. DOI: <https://www.doi.org/10.2478/bvip-2014-0007>
- Jankovsky J, Mabre B, and Gerhold R (2017). Identification of a Novel Renal Coccidian (Apicomplexa: Eimeriidae) from the Great-Horned Owl (*Bubo virginianus*), USA. *Journal of Wildlife Diseases*, 53(2): 368-371. DOI: <https://www.doi.org/10.7589/2016-06-132>
- Kot TF, Dovgiy YY, Rudik OV, Gazaryan VN, and Lebid HV (2020). Pathomorphological changes in individual tubular and parenchymal organs of quails according to eimeriosis. *Veterinary Science Technologies of Animal Husbandry and Nature Management*, 5: 70-75. DOI: <https://www.doi.org/10.31890/vtvp.2020.05.13>
- Kumar YR, Namratha ML, Sawale GK, Ramesh GK, Mahesh B, Ashok Kumar Reddy KB, and Lakshman M (2019). Occurrence of intestinal and caecal coccidiosis in Rajasree birds. *Journal of Animal Research*, 9(6): 875-878. DOI: <https://www.doi.org/10.30954/2277-940X.06.2019.12>
- Lotveld P, Fallahshahroudi A, Bektic L, Altimiras J, and Jensen P (2017). Chicken domestication changes expression of stress-related genes in brain, pituitary and adrenals. *Neurobiology of Stress*, 7: 113-121. DOI: <https://www.doi.org/10.1016/j.ynstr.2017.08.002>
- Mohammad NH (2012). A study on the pathological and diagnosis of *Eimeria* species infection in Japanese quail. *Basrah Journal of Veterinary Research*, 11(1): 318-333. DOI: <https://www.doi.org/10.33762/bvetr.2012.54858>
- Morgan KJ, Alley MR, Pomroy WE, Gartrell BD, Castro I, and Howe L (2013). Extra-intestinal coccidiosis in the kiwi (*Apteryx* spp.). *Avian Pathology*, 42(2): 137-146. DOI: <https://www.doi.org/10.1080/03079457.2013.776665>
- Mulisch M, and Welsch U (2015). *Romeis – mikroskopische technik*. Spektrum Akademischer Verlag, Heidelberg, pp. 382-420. DOI: <https://www.doi.org/10.1007/978-3-642-55190-1>
- Novilla MN, Carpenter JM, Jeffers TK, and White SL (1989). Pulmonary lesions in disseminated visceral coccidiosis of sandhill and whooping cranes. *Journal of Wildlife Diseases*, 25(4): 527-533. DOI: <https://www.doi.org/10.7589/0090-3558-25.4.527>
- Patra G, Rajkhowa T, Ali M, and Tiwari JG (2009). Studies on clinical, gross, histopathological and biochemical parameters in broiler birds suffered from *Eimeria necatrix* infection in Aizawl district of Mizoram, India. *International Journal of Poultry Science*, 8(11): 1104-1106. DOI: <https://www.doi.org/10.3923/ijps.2009.1104.1106>
- Reavill D, and Schmidt R (2019). Post-mortem examination. *Manual of backyard poultry medicine and surgery*. BSAVA. *Manual of Bachyard Poultry Medicine and Surgery*, 25: 291-308. DOI: <https://www.doi.org/10.22233/9781910443194.25>
- Ruff MD, and Allen PC (1982). Changes in liver glycogen of broilers during coccidiosis. *Veterinary Parasitology*, 10(4): 285-295. DOI: [https://www.doi.org/10.1016/0304-4017\(82\)90079-6](https://www.doi.org/10.1016/0304-4017(82)90079-6)
- Sawale GK, Rambabu D, Kommu S, Bhandurige MS, Ramesh G, and Lakshman M (2018). Outbreak of intestinal coccidiosis due to *Eimeria necatrix* in rajasree birds: patho-morphological and electron microscopic study. *International Journal of Livestock Research*, 8(12): 247-251. DOI: <https://www.doi.org/10.5455/ijlr.20180406062457>

- Scanes C (2016). Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poultry Science*, 95(9): 2208-2215. DOI: <https://www.doi.org/10.3382/ps/pew137>
- Seleznev S, Krotova E, and Vetoshkina G (2015). The main principles of the structural organization of the immune system of the Japanese quails. *Journal Agronomy Animal Industries*, 4: 66-73. DOI: <https://www.doi.org/10.22363/2312-797X-2015-4-66-73>
- Shamim A, Hassan M, Yousaf A, Iqbal MF, Zafar MA, Siddique RM, and Abubakar M (2015). Occurrence and identification of *Eimeria* species in broiler rearing under traditional system. *Journal of Animal Science and Technology*, 57(1): 41-46. DOI: [10.1186/s40781-015-0074-0](https://www.doi.org/10.1186/s40781-015-0074-0).
- Sharma S, Azmi S, Iqbal A, Nasirudullah N, and Mushtaq I (2015). Pathomorphological alterations associated with chicken coccidiosis in Jammu division of India. *Journal of Parasitic Disease*, 39(2): 147-151. DOI: <https://www.doi.org/10.1007/s12639-013-0302-9>
- Song H, Liu D, Xu J, Wu L, Dai Y, Liu M, and Tao J (2017). The endogenous development and pathogenicity of *Eimeria anseris* (Kotlan, 1932) in domestic goslings. *Parasitology Research*, 116: 177-183. DOI: <https://www.doi.org/10.1007/s00436-016-5274-0>
- Soutter F, Werling D, Tomley F, and Blake D (2020). Poultry coccidiosis: design and interpretation of vaccine studies. *Frontiers in Veterinary Science*, 7: 101-113. DOI: <https://www.doi.org/10.3389/fvets.2020.00101>
- Spencer K, Evans N, and Monaghan P (2009). Postnatal stress in birds: a novel model of glucocorticoid programming of the hypothalamic-pituitary-adrenal axis. *Endocrinology*, 150(4): 1931-1943. DOI: <https://www.doi.org/10.1210/en.2008-1471>
- Umar HA, Lawal IA, Okubanjo OO, and Wakawa AM (2014). Morphometric identification, gross and histopathological lesions of *Eimeria* species in Japanese quails (*Coturnix coturnix japonica*) in Zaria. *Journal of Veterinary Medicine*, 5: 2-6. DOI: <https://www.doi.org/10.1155/2014/451945>
- Vrba V, and Pakandl M (2014). Coccidia of turkey: from isolation, characterisation and comparison to molecular phylogeny and molecular diagnostics. *International Journal for Parasitology*, 44(13): 985-1000. DOI: <https://www.doi.org/10.1016/j.ijpara.2014.06.004>
- Zaefarian F, Abdollahi M, Cowieson A, and Ravindran V (2019). Avian liver: the forgotten organ. *Animals*, 9(2): 63-86. DOI: <https://www.doi.org/10.3390/ani9020063>