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Pathologic-anatomical Changes in the Comorbidity of Eimeriosis and Tuberculosis in Domestic Chickens and Decorative Pheasants (*Phasianus colchicus* L., 1758)

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ABSTRACT

The study of patho-anatomical changes is essential in identifying pathological processes and diagnosing especially comorbid pathologies. The aim of this study was to reveal pathological changes and differences in the spontaneous comorbidity of tuberculosis (*Mycobacterium avium*) and eimeriosis (*Eimeria* spp.) in adult chickens and ornamental pheasants. The paper highlighted the results of pathological-anatomical changes in adult domestic chickens (n = 17) and ornamental pheasants (n = 5) with spontaneous comorbidity of eimeriosis and tuberculosis. Mycobacteria was detected using bacterioscopy of smears-prints from fragments of organs and *Eimeria* oocysts were detected by the Füllenborn flotation method. In pheasants, tubercular granulomas were found in the intestine, liver, and spleen in combination with scarring and swelling of the wall and mesentery, and venous stasis in the mesenteric vessels. In addition, hematomas and organ destruction in the liver and spleen were found in pheasants. In domestic chickens, tuberculous granulomas and steatosis were found in the liver. In the intestines, there were indications of mucocatarrhal inflammation, edema, hyperplasia, and small hemorrhages in the area of the cecal-intestinal diverticulum. Tuberculous nodules, internal hemorrhages around the perimeter of the tubercle, and devascularization were observed in the spleen. The present study revealed notable differences in the pathological and anatomical changes resulting from the comorbidity of eimeriosis and tuberculosis in domestic chickens and pheasants.

Keywords: Avian tuberculosis, Comorbidity, Eimeriosis, Intestine, Liver, Pathological change, Spleen

INTRODUCTION

In Ukraine, around 50% of chickens are raised in individual auxiliary farms. These farms commonly employ extensive rearing systems and walking areas for joint maintenance of different species and age groups of poultry, which increases the risks of the occurrence and spread of infectious diseases (Liulin, 2023). The existing variability of pathogens and the associative course of diseases or pathologies (comorbidity), especially with a weakened immune response of the body, create certain difficulties in their diagnosis (Campbell-Scherer, 2010; Shin and Shin, 2021). Avian eimeriosis remains a serious problem in poultry production (Dalloul and Lillehoj, 2006) and requires continuous control (Quiroz-Castañeda and Dantán-González, 2015) due to a high prevalence of up to 100% and a mortality of up to 70% in the absence of treatment. Poultry tuberculosis, caused by the bacterium (*Mycobacterium avium*), has a significant impact not only on chickens but also on domestic animals (Polaček and Aleksić-Kovačević, 2016; Komatsu et al., 2017; Lamuka et al., 2018) since the causative agent can induce relevant pathologies in a wide range of species. Moreover, this pathogen poses a serious underestimated threat to human health, especially in an immunodeficient state (Azar et al., 2019; Zhurylo et al., 2020; Kaczmarkowska et al., 2022). Moreover, it causes significant sanitary-epidemiological and social problems due to the possibility of transmission of the causative agent of avian tuberculosis from domestic and ornamental birds to humans (Slany et al., 2016; Patiño et al., 2018; Busatto et al., 2019).

Avian tuberculosis often remains undetected during a person's lifetime, as it typically manifests without noticeable clinical symptoms. It is commonly diagnosed only posthumously, highlighting the challenge of identifying the infection while the individual is alive (Crilly et al., 2021). In cases of avian tuberculosis, the primary diagnostic indicators are the distinctive patho-anatomical changes observed. Unlike mammals, birds primarily develop the tuberculosis complex (the focus of typical pathological and anatomical changes) in the intestine and liver when infected through the alimentary route. In the case of a generalized form of the infection, it can also affect other organs, including the spleen, exhibiting characteristic pathological and anatomical alterations. In this case, granulomas specific to tuberculosis are detected in the organs, the

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intensity of which depends on the level of natural resistance and the ability of the organism to regenerate, which affects the development and prognosis of the disease (Álvarez et al., 2017; Al-hamadawi et al., 2017; Zikovitz et al., 2018). One of the models of comorbidity of tuberculosis in poultry from farms unfavorable for this disease can be its combination with eimeriosis. As noted, mycobacteria can penetrate, survive and persist in the body of protozoa, including *Eimeria*, contributing to the potential simultaneous infection of birds with *Eimeria spp.* and *Mycobacterium* (*M*) *avium* complex (Lande, 2019; Butler et al., 2020). In addition, both diseases (tuberculosis and eimeriosis) have similar clinical symptoms, including general weakness, cyanosis, diarrhea, cachexia, reduction in egg production, and even cessation of egg-laying in sick chickens.

The pathogenic immunosuppressive effect of *Eimeria* on the body of sick chickens and the phenomenon of tropism of *mycobacteria* to immune structures and their ability to cause an immunodeficiency state contribute to the specified comorbidity, which has an applied diagnostic value (Kim et al., 2019; Ioakeim-Skoufa et al., 2020; Zhurylo et al., 2020). During the patho-anatomical diagnosis of eimeriosis and/or tuberculosis in poultry, it is advisable to consider the organs' topographic and morphological features and the presence of lymphoid elements. The localization and nature of lesions of these structures may differ both within the species and in different age groups of domestic, decorative, or wild birds. Moreover, the scale of indicators of patho-anatomical changes in avian tuberculosis needs to be supplemented with new data, which are the results of complex multi-disciplinary studies, and contribute to the improvement of its diagnosis (Özen et al., 2016; Mabelebele et al., 2017).

Thus, in peacocks, morphological changes caused by the causative agent of avian tuberculosis are found in the glandular stomach, which is rich in lymphoid structures (Ciobotaru et al., 2012; Mayahi et al., 2013; Liakhovich and Maslak, 2021). In pheasants, the saturation of the intestinal wall with lymphoid elements leads to the manifestation of specific pathological anatomical changes during the development of tuberculosis in its structures (Kovtun and Harchenko, 2005; Parisa et al., 2019). This determines the high specific sensitivity of pheasants to the causative agent of tuberculosis and, in epizootic centers, threatens their extinction (Álvarez et al., 2017; Lyakhovich et al., 2019). The level of susceptibility of domesticated pheasants to tuberculosis is also affected by their long-term captivity and insufficient solar insolation (Bekele et al., 2018). Vitamin D deficiency contributes to the development of active tuberculosis infection (Babazadeh et al., 2022). The phenomenon of immunosuppression, both for eimeriosis and tuberculosis, provokes manifestations of comorbidity with other diseases or pathologies (Babu and Nutman, 2016; Olmedo-Reneaum et al., 2020).

In the spectrum of liver pathologies of various human genesis, patterns of comorbidity of tuberculosis-protozoan lesions were recorded (Tsyrkunov and Prokopchik, 2018). When typical pathological changes associated with tuberculosis are identified in poultry, it is crucial not to disregard the possibility of a concurrent occurrence of tuberculosis with diseases that resemble it in terms of pathogen localization and/or lesion manifestation. The diagnosis of eimeriosis-tuberculosis comorbidity in poultry and the peculiarities of its patho-anatomical manifestation will expand the informative scale of pathogenetic links and diagnostic indicators of these diseases.

The purpose of the current study was to investigate and classify macroscopic changes in the liver, spleen, and intestines of domestic chickens and decorative pheasants with comorbidities of tuberculosis and eimeriosis.

MATERIALS AND METHODS

Ethical approval

These studies were conducted in compliance with the ethical norms and principles of the requirements of the European Union.

Pathological and anatomical analysis

A total of 22 dead birds were subjected to a comprehensive pathological and anatomical examination. The sample consisted of 17 chickens, including 7 female Rhode Island breed, 5 female Leghorn breed, and 5 roosters of the *Adler silver* breed aged between 2 and 3 years. Additionally, the study involved 5 adult ornamental pheasants, comprising 3 males aged 2.5, 4.5, and 6 years, as well as 2 females aged 1.5 and 2.5 years. Autopsies of bird carcasses were performed at Department of Normal and Pathological Morphology of the Kharkiv State Zooveterinary Academy, Ukraine (now the State Biotechnological University, Ukraine) by eviscerating the supine position (Dobin and Kokurichev, 1963), following the method of anatomical preparation of the liver, spleen, intestinal tube and its mesentery. To ensure proper preservation, the corpses of the deceased birds were transported from the auxiliary farms and the ecopark of the Kharkiv region in special thermal containers with ice, maintaining a temperature of +4°C within 12 hours after the birds' death.

Coproscopic analysis

A small amount (the size of a pea) of the permeable intestine (chyme) from different parts of the intestine was carefully mixed with 1 ml of 50% water-glycerol solution in a chemical beaker. Then, a drop of this solution was applied to a glass slide, covered with a coverslip, and subjected to microscopy (magnification ×80 and ×400) to identify *Eimeria* oocysts and their development stages.

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Flotation method

Eimeria oocysts are detected by the Füllenborn flotation method. To do this, samples of feces (3 g) were taken from the end part of the intestine (cloaca) of bird corpses, which were mixed with 30 ml of a saturated NaCl solution and filtered through a metal filter (hole size 0.8 - 1.0 mm). The filtrate was settled for 30 minutes, after which a metal loop (diameter 0.8 cm) was used to take samples from the liquid surface of the studied samples. Specifically, 3 drops of the surface film were collected and transferred to a glass slide and microscopically examined for the presence of *Eimeria* oocysts. The number of oocysts in 1 gram of feces was determined using Goryaev's kamera (Halat et al., 1999). The species affiliation of pathogens of *Eimeria spp.* was determined based on morphological examinations, including the analysis of oocyst shell shape, color, presence or absence of micropyle, polar granules, and residual body in both oocysts and sporocysts. Additionally, their biological characteristics such as sporulation periods, length of prepatent and patent periods were considered. The obtained results were then compared with the data from determination tables, specifically those outlined by Pellerdy (1974).

Microbiological analysis

Mycobacteria were detected by bacterioscopy of smears-prints from fragments of organs affected by granulomas: liver, intestinal wall, and spleen according to the Ziel-Nielsen method (Asmolov, 2002).

Statistical analysis

The obtained results were analyzed using Statistica 10 software (StatSoft Inc., USA). Differences between the parameters of the experimental groups were determined using ANOVA. The test Duncan was used to determine the p value. The results were presented as the mean ± standard error of the mean (M ± SE). The difference between groups was considered significant at $p < 0.05$.

RESULTS

Based on patho-anatomical changes and the results of microscopy of samples of domestic chickens and decorative pheasants, the comorbidity of eimeriosis and tuberculosis was established. The causative agents were identified as *Eimeria*. Bacterioscopy of smears-imprints taken from affected areas of the liver, intestinal tube walls, and spleen was performed. The smears were stained using the Ziel-Nielsen method. The examination revealed the presence of red acid-resistant mycobacteria, which appeared as rod-shaped bacteria either singly or in pairs.

The pathological examination of pheasant corpses

The carcasses of the investigated male pheasants were, to varying degrees, exhausted (with skeletal muscle atrophy), dehydrated, and with signs of general anemia. The carcasses of female pheasants were moderately fat with anemia. Macroscopically, pheasants showed changes in the liver, spleen, intestinal tube, and mesentery.

The intestinal tube was deformed to varying degrees due to asymmetrically located spherical thickenings in the form of granulomas of different diameters and degrees of maturity, which were visible from the side of the highly moist and shiny serous membrane. In male pheasants, the sections of the intestinal wall between the granulomas were wrinkled. The intestinal tube was slightly shortened (without characteristic anatomical bends, Figure 1). The lumen of the intestinal tube was empty. On a cross-section in most areas, it was narrowed and deformed due to transmural granulomas, and individual post-traumatic ulcers with diffuse hemorrhages (after the destruction of granulomas) were observed. Blood stagnation in the mesenteric venous vessels was observed. The blood was a dark cherry-colored, asphyxial type (lightens on contact with air).

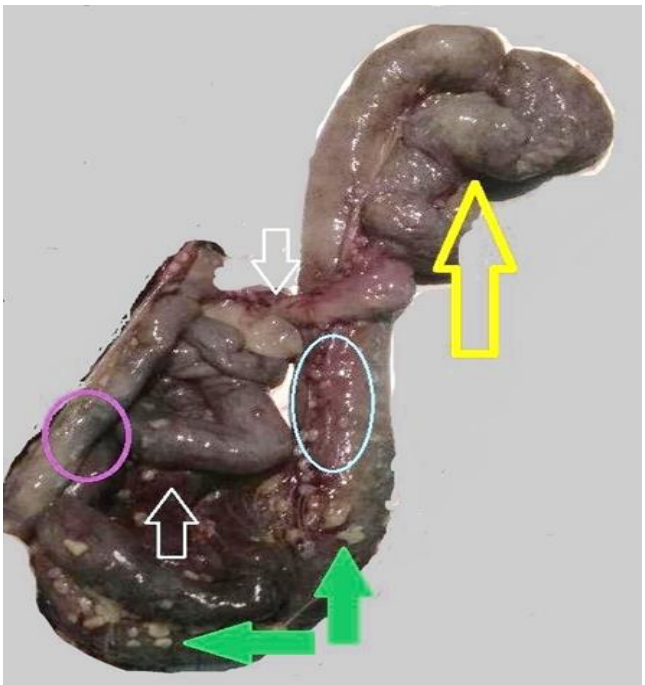

Figure 1. Macroscopic intestinal lesions of a decorative pheasant with comorbidity of eimeriosis and tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2019. **Yellow arrow**: The deformation of the wall, **Green arrows**: Multiple light yellowish granulomas, **Purple oval**: The increased gloss of the serous membrane due to edema, **Blue oval**: Edema of the mesentery, **White arrows**: venous stasis in mesenteric vessels

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In female pheasants, with the use of low-multiplier optical lenses, isolated small whitish nodules were found on the side of the serous membrane of the jejunum; on the mucous membrane in different parts of the intestinal tube - partial redness, swelling, individual small hemorrhages according to the type of ecchymoses. Male pheasants were diagnosed with total destructive tuberculous jejuno-ileo-typhlitis (inflammation of the walls of the jejunum, ileum, and cecum). In female pheasants, local granulomatous tuberculous jejunitis combined with edematous-hemorrhagic enteritis was classified.

Multiple granulomas of different diameters and degrees of maturity were found in the liver of male pheasants, including one with a central area of caseous necrosis (Figure 2). Signs of tuberculous hepatitis and atrophy of the organ were observed in the liver of female pheasants (sharp edges, wrinkling of the capsule and light pink-gray color in the areas between yellowish granulomas of different diameters, weak vascularization, Figure 3). The spleen of female pheasants was slightly enlarged due to the presence of small nodules of light gray color (Figure 4, classified productive miliary splenitis).

In male pheasants, the spleen was difficult to differentiate due to extreme deformation and destruction. It revealed confluent large (0.5-0.7 cm) gray-white nodules with caseous content and spherical-ellipsoid formations of dark brown-cherry color (0.5-0.8 cm) with an area of damage up to 30-45% ($p < 0.05$) of the surface (with decay masses soaked in dark cherry blood). Tuberculous granulomas and subcapsular hematomas were detected. Coproscopic examinations conducted on samples obtained from the intestinal tubes of the examined pheasant carcasses revealed a 100% infestation rate by *Eimeria*. The intensity of invasion was 46.7 ± 3.9 - 85.3 ± 6.4 oocysts per gram of feces by the species: Eimeria colchici (Norton, 1976), *Eimeria phasiani* (Tyzzer, 1929) and *Eimeria duodenalis* (Norton, 1976), respectively 24, 7%. 63.4% and 11.9% of the total number of oocysts ($p < 0.05$).

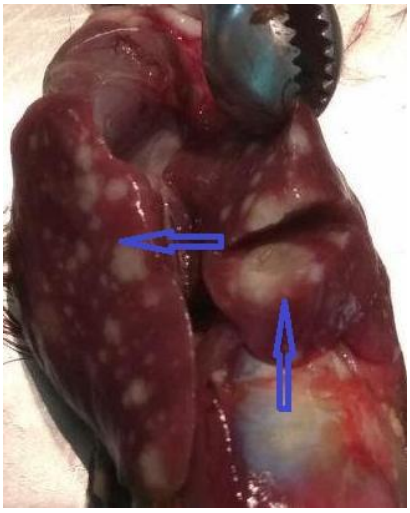

Figure 2. Macroscopic lesions of the liver of a decorative pheasant with comorbidity of eimeriosis and tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2019. Multiple miliary and mature large focal granulomas (arrows).

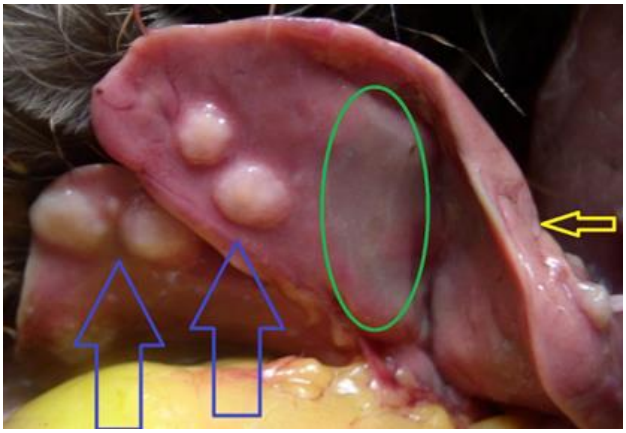

Figure 3. Macroscopic lesions of the liver of a female pheasant with comorbidities of eimeriosis and tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2019. The isolated large focal granulomas (**blue vertical arrow**); signs of atrophy (too sharp edges of the organ and deformed surface, **yellow arrow**); devascularization of the liver (presence of bloodless grayish areas, **green oval**).

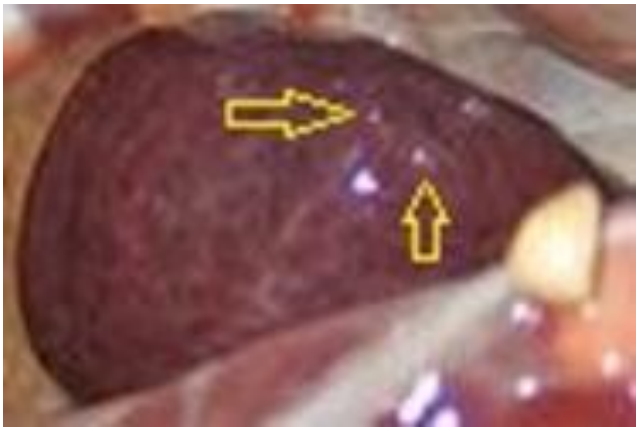

Figure 4. Spleen of a pheasant with concomitant diseases of eimeriosis and tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2019. An increase in the volume of the organ due to small light gray granulomas (**yellow arrows**).

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Results of pathological examination of chicken carcasses

According to the results of the pathological examination of the carcasses of domestic chickens (12 females and 5 roosters), the poultry had different levels of fatness. During the examination of the organs in the thoracic and abdominal cavity of chickens, typical changes indicative of tuberculosis were observed. These changes manifested as specific granulomas present in the liver, intestinal wall, and its mesentery. At the same time, in three out of seven chickens, multiple tubercular nodules in the liver were combined with individual specific miliary granulomas in the intestinal wall (their visualization was facilitated by the use of low-optic lenses). Destructive granulomatous miliary, small-focal, and large-focal hepatitis with the destruction of the capsule and parts of the organ was diagnosed in the liver of the examined chickens (Figure 5).

In the first group of chickens, the livers of five exhibited fragility upon removal from the thoracic cavity, lacking clear differentiation of the capsule. These livers displayed multiple specific tuberculous nodules of varying diameters, characterized by a light-yellowish color. These nodules were observed as miliary and small foci. Additionally, there were indications of steatosis, with the organ appearing enlarged due to capsule rupture. The color of the liver exhibited variation, with light beige areas between the nodules and pale yellowish nodules themselves. During the liver sectioning process using a scalpel, fatty deposits were left on the blade, and light yellowish caseous formations were discovered within the organ (Figure 6).

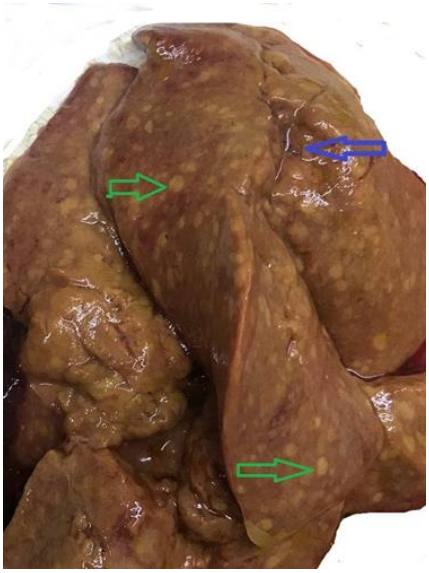

Figure 5. Macroscopic lesion of the liver of a domestic chicken (Leghorn breed) eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. Multiple miliary and small-focal granulomas of light yellowish color under the capsule (**green arrows**) and in the thickness of the organ (**blue arrow**). Kharkiv region, Ukraine, 2020.

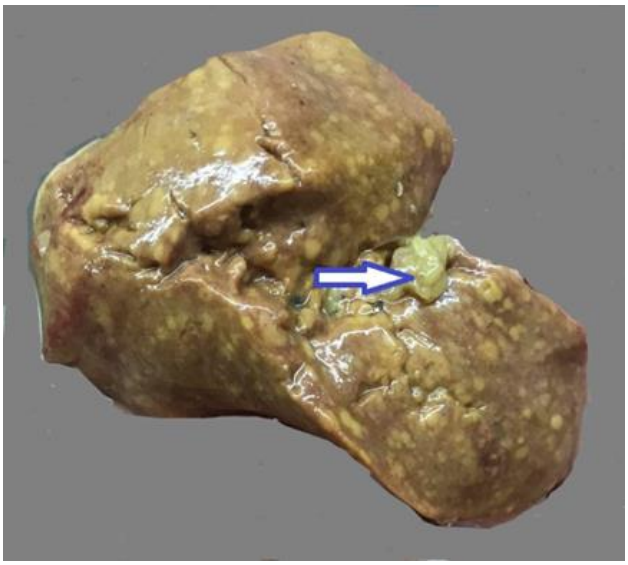

Figure 6. A fragment of the liver of a domestic chicken (breed - Leghorn, age 2.5 years) for comorbidities of *Eimeria* and tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. Macroscopic lesions of the liver: Multiple granulomas of pale-yellow color, rupture of the capsule and parenchyma of the organ, caseous substrate in the area of the rupture (**arrow**).

Coproscopic examinations of the intestinal tube material from the chicken carcasses revealed the presence and identification of various *Eimeria* species. These included *Eimeria acervulina* (Tyzzer, 1929), *Eimeria brunetti* (Levine, 1939), *Eimeria maxima* (Tyzzer, 1929), *Eimeria mitis* (Tyzzer, 1929), *Eimeria necatrix* (Jonson, 1927), *Eimeria praecox* (Jonson, 1927), and *Eimeria tenella* (Raillet and Lucet, 1891). These species accounted for 18.6%, 7.6%, 16.5%, 4.9%, 11.3%, 2.4%, and 38.7% ($p < 0.05$) of the total number of oocysts, respectively. The intensity of invasion ranged from 140.6 ± 4.8 to 263.2 ± 5.7 oocysts per gram of feces.

With the help of low-optical lenses, single whitish miliary granulomas with subserosal localization were observed in the intestinal wall of three chickens (within the topographic boundaries of the jejunum, Figure 7). In addition to the mentioned changes, some miliary and small nodular tuberculous nodular lesions of the intestinal mesentery, had elongated ellipsoid shape and pale pink shade (Figure 8). In individuals of roosters and chickens, diffuse hemorrhages were detected from the side of the serous membrane in the intestinal wall, particularly the duodenum, due to the use of low-optical lenses (Figure 9).

Significant changes were observed in the caecum of the examined domestic roosters. These changes included the presence of stagnation, accumulation of exudate masses, detritus, and gases. As a result, a local flask-like expansion and stretching of the intestinal wall occurred. In certain areas, the intestinal wall became thin to the point of translucency, which could be attributed to the desquamation of the epithelium (Figure 10). Hyperplasia of the cecal tonsils (tonsils), swelling, mass of exudate and/or decay were found in part of the studied chickens at the level of the mucous membrane

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in the area of the cecal diverticula (Figure 11). Brown-red masses of mucous-hemorrhagic exudate and disintegration of the intestinal wall were found in individual chickens in the lumen of the caecum (Figure 12).

According to the sectional examination of the intestine in chickens, lesions of two categories were established, including non-intense productive (granulomatous) subserous enteritis within the jejunum, characteristic of tuberculosis with intestinal localization of changes, and intense edematous-hemorrhagic and/or desquamative typhlitis (in some individuals) or sectoral moderate duodeno-jejuno-ileo-typhlitis (in the majority of examined chickens), typical for poultry eimeriosis.

Thus, the obtained results of studies of pathoanatomical changes in the intestine and their classification according to the comorbidity of tuberculosis/eimeriosis in chickens and pheasants can serve as diagnostic markers (Table 1). Except for one rooster, the spleen of the studied domestic chickens had a preserved shape, a moderately increased volume, and a uniform color (from light cherry to dark cherry brown in different individuals, Figure 13). An enlargement of the spleen due to hyperplasia was observed. In some individuals, the enlargement was also associated with venous stasis. Importantly, the integrity of the spleen's capsule remained preserved throughout the examination. In one examined rooster, the spleen was deformed due to the presence of multiple yellowish nodules (0.2-0.4 cm in size), moderately enlarged, unevenly colored, both from the surface and on the section (there were yellowish nodules on a devascularized light cherry background, the perimeter of some of which, with the help of low-power optical lenses, perifocal hemorrhages of bright red color were visualized, Figure 14).

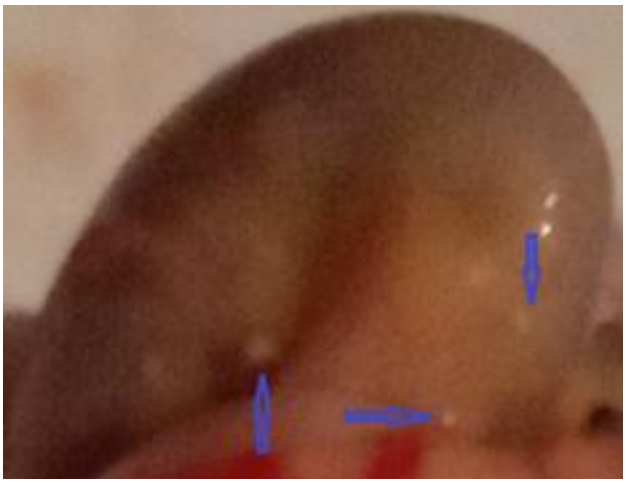

Figure 7. Macroscopic lesions of the wall of the jejunum of a domestic chicken (Leghorn breed) due to the comorbidity of eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. Miliary granulomas from the side of the serous membrane (**arrows**). Magnification with a small optical lens × 5.

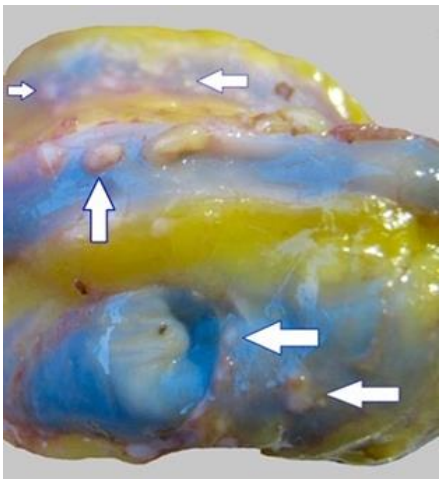

Figure 8. Lesions of the intestinal mesentery of a domestic chicken (Rhode Island breed) due to the comorbidity of eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. The presence of miliary and small-focal ellipsoidal granulomas of pale pink color (**arrows**).

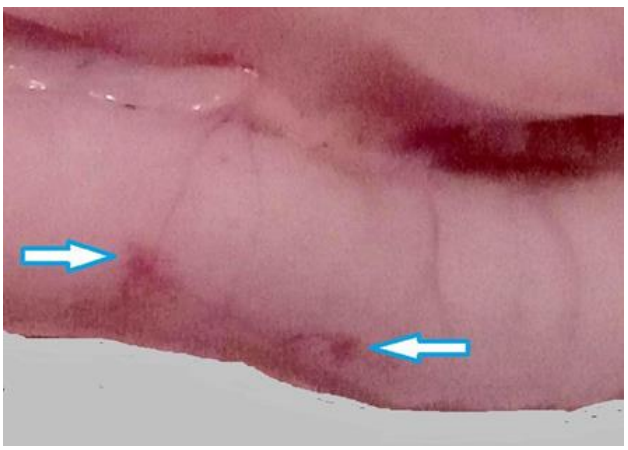

Figure 9. A fragment of the U-shaped knee of the duodenum and pancreas of a domestic rooster (*Adler silver* breed) with comorbidity of eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. Diffuse subserosal hemorrhages (arrow). Magnification with weak optical lens × 2.

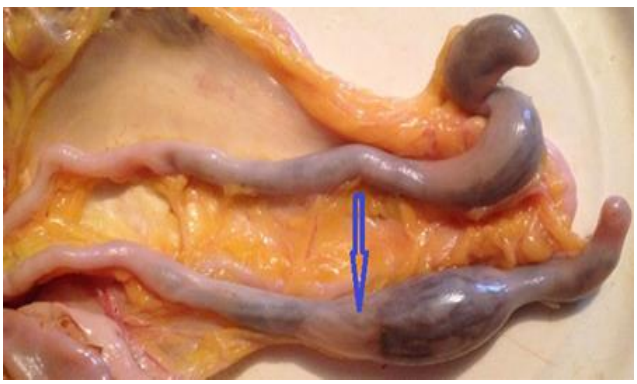

Figure 10. Caeca lesions (caeca) of a domestic rooster (*Adler silver* breed) with comorbidities of *Eimeria*/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. Bulbodental expansion and thinning of the wall (arrow). Magnification with a low-optical lens × 3.

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Figure 11. Cecal diverticulum of a domestic rooster (*Adler silver* breed) with comorbidity of eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. hyperplasia of the lymphoid tonsils (**arrow**), swelling, and masses of exudate on the surface of the mucous membrane. Magnification with a low-optical lens × 5.

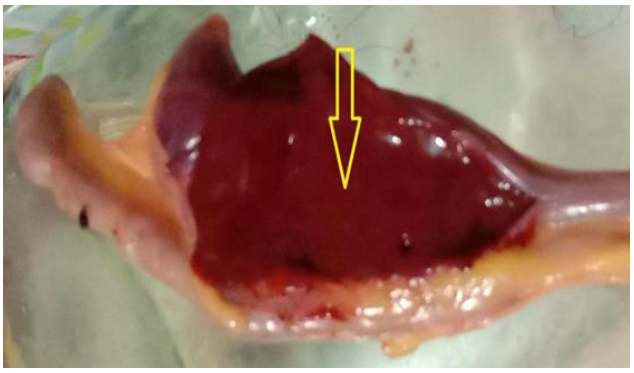

Figure 12. Damage to the caecum of a domestic chicken (Rhode Islan breed) due to comorbidity of eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. The presence of masses of mucohemorrhagic exudate and decomposition products in the lumen (**yellow arrow**).

Table 1. Comparative characteristics of macroscopic indicators of intestinal lesions of domestic chickens and decorative pheasants under the comorbidity of eimeriosis /tuberculosis

Index	Domestic chickens	Decorative pheasants
Swelling of the intestinal wall	Local	Total
Hemorrhages	Diffuse subserous and small (according to the type of ecchymoses): In the serous and mucous membranes of the duodenum (due to invasion by *Eimeria acervulina*), jejunum (for *Eimeria maxima* and *Eimeria necatrix* infestations), in the mucous membrane of cecum and their diverticula (in case of *Eimeria tenella* infestation).	Diffuse post-traumatic in the area of transmural tuberculous ulcers; in the mucous membrane of the duodenum, jejunum for infestations (*Eimeria duodenalis* and *Eimeria phasiani*), caecum (*Eimeria colchici*)
Granulomas	Single, small (miliary), spherical, white, in the wall of the jejunum (visualized using low-optical lenses)	Different diameters (small and large foci) and degrees of maturity (including a light yellowish central zone of caseous necrosis) in the wall of the jejunum, ileum, and cecum
Deformation of the wall	Local, within the granulomas in the wall of the jejunum (visualized using low-optic lenses)	Total, due to granulomas and scarring (in males), local in female pheasants due to granulomas in the wall of the jejunum

Figure 13. Spleen of a domestic cockerel (*Adler silver* breed) with comorbidities of eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. The capsule tension (enlargement of the spleen); venous congestion (**yellow arrow**).

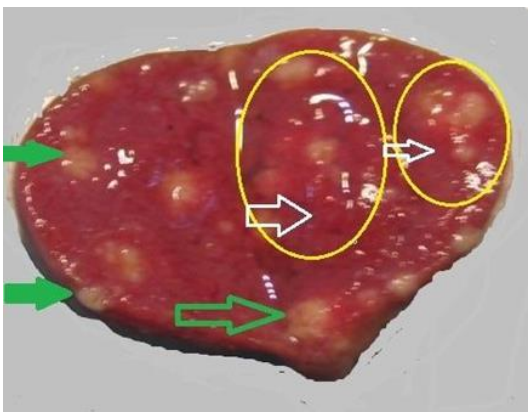

Figure 14. The spleen of a domestic cockerel (*Adler silver* breed) with comorbidities of eimeriosis/tuberculosis. The sample was collected from Kharkiv region of Ukraine, 2020. Tuberculous nodules (**green arrows**), internal hemorrhages around the perimeter of tubercular nodules (**white arrow**), white triangle in **yellow ovals**; the area of devascularization is also shown.

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DISCUSSION

In the context of comorbidity between tuberculosis and eimeriosis, the findings of the current study indicated characteristic signs of specified diseases. However, there were certain species differences between domestic chickens and ornamental pheasants. In the studied pheasants, the patho-anatomical changes were characterized by the dominance of pronounced, more intense damage to the intestinal wall with the predominance of atrophic changes in its structures, which characterized the duration of the lesions and the previous strength of the body's protective. In particular, the presence of lymphoid anatomic and morphological structures of the intestinal wall determined the peculiarities of the manifestation of pathological changes, which was also mentioned by other researchers (Kovtun and Harchenko, 2005; Parisa et al., 2019).

In lymphoid formations there were primary foci of struggle with *M. avium* were formed in the intestinal wall of pheasants (tuberculous granulomas), which appear in other organs due to the generalization of the pathogen, which is confirmed by the data (Zikovitz et al., 2018). The degree of tuberculous granulomas of the intestinal wall in pheasants differed between males and females. Thus, in male pheasants, lesions of the intestinal wall were total and transmural, which indicates their duration. In female pheasants, only isolated tubercular nodules were found on the serous membrane, and moderate superficial lesions were caused by *Eimeria spp.* (infestation intensity of 46.7 ± 3.9-85.3 ± 6.4 oocysts per gram of feces), which was consistent with a study by Goldová et al. (1998). In the intestines of the examined chickens, changes typical for inflammation were detected, in particular, damage to the surface layers of the mucous membrane (exudative with the involvement of lymphoid structures and manifestations of hyperplasia), which were caused by the action of pathogens *Eimeria spp.* (83.7 ± 11.6-161.8 ± 23.8 oocysts in 1 gram of feces), which was supported by Liulin et al. (2021), indicating similar variants of pathological changes in the intestines of pigeons with experimental eimieriosis. Kot et al. (2020) also observed signs of catarrhal-serous, catarrhal-mucous, and hemorrhagic enteritis, as well as necrosis of the structures of the intestinal mucosa, during eimeriosis in quails. The main patho-anatomical changes due to the comorbidity of tuberculosis/emmeriosis in the studied chickens were found in the liver. The degree of intestinal damage was less pronounced in chickens than in pheasants.

Thus, during spontaneous alimentary infection of pheasants with pathogens of *M. avium* and *Eimeria* spp., lymphoid components of the intestinal wall were initially damaged. The patho-anatomical changes indicated the chronic nature of pathological processes due to the possibility of remissions. At the same time, repeated cases of such infection caused total damage to the intestinal wall in pheasants, and there was a combination of signs of its tuberculous productive inflammation and atrophy due to chronic lesions and the inability to regenerate the lost structures fully. Consequently, these pathological changes resulted in spasms and wrinkling of specific segments of the intestine, attributed to scarring processes. These alterations led to narrowing, deformation, and wrinkling of the affected areas, ultimately causing the absence of chyme within the intestinal lumen. This observation aligns with the findings reported by Lyakhovich et al. (2019). The specified changes in male pheasants led to impaired function of the affected parts of the intestine, development of dehydration, general cachexia and anemia due to subcapsular hematomas of the spleen and intestinal bleeding in concomitant diseases (*Eimeria*/tuberculosis). Lyakhovich et al. (2019) indicate the differences in the manifestations of patho-anatomical changes due to tuberculous lesions in ornamental pheasants, the influence of their diet on their development, and background steatosis of the liver. The current study indicated a slightly different pattern of combined changes in the liver of chickens. Tuberculous lesions and signs of steatosis were approximately the same in severity and were easily visualized. This is explained by the higher content of lipids in the liver of domestic chickens, which in case of steatosis, contributed to the development of mycobacteria and the manifestation of the corresponding patho-anatomical tuberculosis lesions of the organ.

In case of granulomatous-necrotic tubercular hepatitis of pheasants (Curland et al., 2018), concomitant pathology (23.1%) of *Eimeria* was also detected (which was confirmed by parasitological studies) with the presence of *Eimeria* oocysts in the intestines. At the same time, granulomatous lesions of the liver were expressed to a lesser extent, compared to the changes detected in the intestine. However, it was not indicated whether the same individual pheasants simultaneously had tuberculous pathological changes and *Eimeria* invasion. *Eimeria* invasion of pheasants is quite widespread in Europe. Researchers have coproscopically detected *Eimeria spp.* (in 64% of 2-week-old pheasants and 73% of 8-week-old pheasants, Goldová et al., 2006). Gassal and Schmăschke (2006) diagnosed *Eimeria* infestation among 41% of adult pheasants with low intensity by three species, including *Eimeria phasiani*, *Eimeria duodenalis* and *Eimeria tetartooimia* (at the same time). The species *Eimeria tetartooimia* was discovered for the first time in Germany, although it is widespread in the Japanese green pheasant and is found in zoos in Japan). A total of 10 *Eimeria* species have been morphologically and molecularly identified and described in pheasants, as reported by Matsubayashi et al. (2021). Notably, three of these species, namely *Eimeria colchici*, *Eimeria duodenalis*, and *Eimeria phasiani*, have been found to have a close association with tuberculosis according to researchers from the United Kingdom. In the examined carcasses of chickens and pheasants, a simultaneous infection (comorbidity) of *Eimeria* spp. and *M. avium* was diagnosed (Norton, 1976).

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Similar pathologies in pigeons are described by Dong et al. (2018) and indicate the simultaneous detection of two to five species of *Eimeria*. The localization and degree of damage to the intestinal wall in different parts depend on the species composition (Kot et al., 2020). In particular, inflammation of the caecum caused by *Eimeria tenella* leads to their expansion, violation of evacuation ability, and stagnation of contents (chyme, inflammatory exudate, masses of detritus and gases) favors the development of pathogens in their lumen, and the creation of anaerobic conditions in particular, *Clostridium perfringens* and *M. avium* (Stanley et al., 2014; Macdonald et al. 2019; Michalska et al., 2021). This finding aligns with a recent study by Madlala et al. (2021), which demonstrated that *Eimeria* spp. parasitization disrupts the homeostasis of the intestinal microflora, leading to an imbalance. This imbalance, in turn, promotes the proliferation of clostridia bacteria. Notably, the presence of *Eimeria tenella* has been shown to facilitate the reproduction of potentially pathogenic bacteria (Chen et al., 2020). Moreover, this disruption of the intestinal microflora increases susceptibility to infectious diseases, including tuberculosis (Curland et al., 2018). The comorbidity of eimeriosis and tuberculosis in pheasants was combined with lesions of the surface layers of the intestinal wall by *Eimeria*, and its deeper layers, especially its lymphoid structures, by tuberculosis pathogens (the wall of pheasants' intestines is saturated with a significant number of lymphoid structures). When the lymphoid structures of the intestinal wall are destroyed due to infection with *M. avium* and *Eimeria spp.*, it leads to water absorption disorders, the development of severe dehydration of the body, and the death of pheasants (Yevstafieva and Kovalenko, 2019). Thus, it is appropriate to consider the caecum of pheasants as a kind of niche for the reproduction of mycobacteria. The simultaneous development of *Eimeria* in them creates prerequisites for infection, which is confirmed by Liou et al. (2001), while immunization of pheasants with a low dose of oocysts *Eimeria colchici* protects them from mass death and potentially prevents the development of anaerobic infections and tuberculosis.

The death of domestic chickens (unlike pheasants) is related to liver dysfunction, namely the development of cytolytic syndrome. A similar syndrome also leads to developing tuberculous liver damage in humans (Okusok et al., 2017). Polymorbidity is a combination of liver lesions of different genesis in humans, particularly tuberculous and protozoal (Tsyrkunov and Prokopchyk, 2018). Combined liver lesions in domestic chickens (tuberculous and steatosis) have been reported (Liakhovich et al., 2022). Miliary nodules and diffuse or petechial hemorrhages in the liver and/or spleen and intestines were found in domestic chickens that died from tuberculosis (Debelu et al., 2022).

Pathological signs of duodeno-jejuno-ileo-typhlitis were diagnosed in the intestines of most chickens in the current study. According to Yatusevich et al. (2016), the specified structural changes in the intestinal wall of chickens should be considered morphological markers of eimeriosis since certain species of its causative agents have their own species-specific localization in the intestines. During the spontaneous invasion of Japanese quails by *Eimeria tsunodai*, morphological changes were detected in the caeca. The degree of their damage increased with the age of the bird. It was characterized by the destruction of the mucous membrane of the caeca, which led to atrophy of the folds and degradation of the mucous membrane, accompanied by the development of necrotic enteritis. It was accompanied by the loss of body weight of the quails and dehydration (Gesek et al., 2015). A similar picture was also observed in the studied pheasants, which were diagnosed with dehydration and general cachexia, manifested by skeletal muscle atrophy.

Both typical tuberculous lesions and their combination with natural complications (decomposition nodes and hemorrhages/hematomas) were diagnosed in the spleens of the studied pheasants and chickens. Critical pathologies in pheasants were subcapsular hematomas of the spleen, its destruction with devascularization, and the development of regular hemorrhagic shock. Combined tuberculous lesions of the spleen and its hematomas were dominant in the studied pheasants. The indicated massive damage to the spleen together with intestinal pathology played a decisive role in the death of the pheasants. In general, pathological changes in organs (liver, intestines, and spleen) were important criteria for diagnosing comorbidity of tuberculosis and eimeriosis in poultry. In pheasants, the nature of the detected changes in visceral organs was characterized by the dominance of specific tuberculous changes in the intestines and spleen, while in chickens, changes in the spleen were recorded in 5.8% and were not as critical as in pheasants. Liver failure was dominant in the mechanism of death of chickens. Manifestation of pronounced tuberculous pathological changes in the liver of female chickens was facilitated by its background damage - fatty hepatosis provoked by a high-energy diet. Signs of steatosis were clearly visualized, which affected its color in the areas located between specific tubercular nodules. It is known that the color of the liver in birds depends, in particular, on the accumulation of lipids that enter the body with feed and as a result, give the organ a yellowish tint (Al-hamadawi et al., 2017).

In the studies of domestic chickens, the background color of the liver was light beige precisely because of excessive lipid infiltration, which is consistent with other studies (Kälsch et al. 2011; Zaefarian et al. 2019). The results of the postmortem examination of chickens and pheasants that died due to comorbidities of *Eimeria*/Tuberculosis were determined. Among decorative pheasants, age is considered a significant factor as the risk of infection by potential pathogens tends to increase with age. On the other hand, in domestic chickens, the risk of liver damage caused by *Eimeria* and tuberculosis pathogens is heightened due to the earlier onset of steatosis, which is attributed to a high-energy diet.

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CONCLUSION

The results revealed significant differences in pathological changes in pheasants and domestic chickens affected by the comorbidity of eimeriosis and tuberculosis. In pheasants, results indicated intestinal damage, tuberculous oviductal ileotyphitis, edematous-hemorrhagic enteritis, cicatricial narrowing, and deformations. Signs of atrophy, miliary, and isolated large-focal tuberculous granulomas were observed in the liver. The spleen demonstrated tuberculous granulomas and subcapsular hematomas. In domestic chickens, the primary lesion was localized in the liver, characterized by destructive granulomatous miliary, small-focal and large-focal hepatitis, accompanied by steatosis and damage to the capsule and parenchyma of the organ. The intestine showed local single miliary tuberculous granulomas and changes of *Eimeria* genesis, mucous-catarrhal enteritis, edema, hyperplasia with small hemorrhages. In the spleen, tuberculous nodules with peripheral hemorrhages and devascularization were found. These findings highlight the need for further research on the pathogenesis and pathoanatomical pattern of comorbidity and polymorbidity of infectious and invasive diseases of domestic and exotic birds.

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Availability of data and materials

All data from the current study is available by request.

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Author's contributions

Petro Lulin conducted a coproscopic study and described its results. Lyubov Lyakhovych conducted the pathological examination, took photographs, described and analyzed the data. Mykola Bogach conducted and described the morphometry of eimeriosis pathogens. Anastasia Ulyanytska participated in patho-anatomical autopsies, conducted microbiological studies, bacterioscopy, and identification of the causative agent. Petro Lyulin, Lyubov Lyakhovych and Mykola Bogach wrote the manuscript. All authors reviewed and approved the final version of the manuscript for publication in this journal.

Competing interests

There are no stated conflicts of interest by the authors.

Ethical considerations

All authors reviewed the manuscript for ethical issues such as plagiarism, consent to publish, misconduct, forgery and/or falsification of data, duplicate publication and/or submission, and redundancy.

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