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Macromorphological Changes after Spontaneous Co-invasion of Eimeriosis, Histomonosis, and Trichomoniasis in Domestic Chickens

Petro Liulin[1]*, Mykola Bogach[2], Liubov Lyakhovich[3], Olena Birka[4], and Alla Petrenko[5]

[1,3,4,5] *State University of Biotechnology, 44, Alchevskikh Str., Kharkiv, 61002, Ukraine*
[2]*Odessa Research Center, National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" Nacional Academy of Agrarian Sciences of Ukraine, Odessa, Ukraine*

*Corresponding author's Email: liulinpetr@gmail.com

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ABSTRACT

The study of macro morphological changes is important for recognizing pathological processes and diagnosing diseases, especially co-invasions. The current study aimed to reveal macro morphological changes during spontaneous co-invasion of *Eimeria* spp., *Histomonas meleagridis*, and *Trichomonas gallinae* in adult chickens. The methods of evisceration and parasitological studies of the carcasses of adult domestic chickens (n = 31) aged 1.5-2.3 years, and (n = 8) are the control group, died of a natural death from farms in the Kharkiv region of Ukraine revealed the peculiarities of manifestations of macro morphological changes in organs, which were characterized by manifestations of pathologies in 100% of cases in dead chickens by changes in the intestines and liver, in 48.39% in the spleen, in 16.13% in the bursa of Fabricius, in 16.13% in the peritoneum and 9.68% in skin. In particular, a mucosal-hemorrhagic inflammatory process was observed in the intestines of chickens with damage to both thin and thick parts (duodeno-jejuno-ileo-typho-cloacitis). This inflammatory process was observed in combination with necrotic-granulomatous lesions on the intestinal wall. A combined lesion of the liver was detected - hepatomegaly on the background of steatosis, multifocal necrosis, and granulomas (shiny, vitreous, with a white center) in the presence of *Histomonas meleagridis* and *Trichomonas gallinae*. A granulomatous splenitis was diagnosed in the spleen of chickens. Necrotic-granulomatous lesions leading to cyst formation were identified in the bursa of Fabricius. Additionally, granulomatous lesions originating from trichomoniasis were observed on the peritoneum and skin. These findings highlight the systemic nature of pathological-anatomical changes resulting from the co-infection of eimeriosis, histomoniasis, and trichomoniasis in domestic chickens. This systemic manifestation signifies the occurrence of multi-organ failure and holds valuable diagnostic implications.

Keywords: Comorbidity, Eimeriosis, Enterohepatitis, Histomonosis, Pathological changes, Trichomoniasis

INTRODUCTION

Poultry plays an important role in providing people with food, production, and supply of animal protein, such as eggs and meat (Bogosavljevic-Boskovic et al., 2010). More than 102.9 million tons of chicken meat are produced annually in the USA (2020 data) with a growing trend (USDA). The efficient functioning of poultry farming is currently facing significant challenges, with various causative agents of poultry diseases posing a threat to food security. Notably, the factors contributing to what is often referred to as "technological diseases," including eimeriosis, play a significant role in these challenges (Godfray et al., 2010). Avian emeriosis is widespread in all countries, so it remains a serious problem at the global level and requires constant control (Quiroz-Castañeda and Dantán-González, 2015). In terms of importance, this disease is among the top three, causing annual losses of more than 14.5 billion US dollars (Dalloul and Lillehoj, 2006; Blake et al., 2020). Millions of dollars are spent on measures to combat *Eimeria* in birds (use of coccidiostats). The ongoing requirement for continuous monitoring and preventative measures, such as vaccination and the elimination of *Eimeria* oocysts from the external environment, remains crucial. Despite these extensive efforts, it is important to acknowledge that the challenge posed by *Eimeria* has yet to be fully resolved (Blake et al., 2020). The use of anti-eimeriosis vaccines is accompanied by high risks of induction of subclinical Eimeriosis (Lee et al., 2022). In addition, in the absence of growth promoters, live vaccines may increase the incidence of bacterial and protozoan enteritis in poultry (Lee et al., 2022). Recently, cases of polymorbid pathologies have become more frequent in chickens (Clarke et al., 2017; Yevstafieva and Kovalenko, 2019). In many countries of the world, instances of eimeriosis, histomoniasis, and trichomoniasis have been observed in adult chickens. This trend has become particularly pronounced following the ban on the use of nitroimidazoles, nitrofurans, and arsenic-containing drugs (CEC, 2002; Hess et al., 2015) since the absence of these drugs led to increased cases of diarrhea, enterohepatitis, often of histomonad and trichomonad etiology, as well as protozoal liver lesions in chickens (Araújo et al., 2015; Dolka et al., 2015; Lopes et al., 2022). However, it should be

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noted that histomonosis and trichomonosis in laying chickens are more often chronic, mild, or even asymptomatic (Beer, et al., 2022). For this reason, adult chickens are usually not suspected of having protozoa (Eimeriosis, histomoniasis, trichomoniasis) during their lifetime since they clinically look healthy or have light, inconspicuous lesions limited to the oral cavity and minor disturbances in the activity of the intestinal canal (Saikia et al., 2023). However, the pathogen can persist for a long time in a flock of laying hens and create a reservoir of invasion (Sigmon et al., 2019). The disease is accompanied by a general weakness of chickens, a decrease in productivity (laying and live weight gain), the death of up to 20% of the flock, and is often diagnosed only postmortem in the presence of characteristic pathological changes and detection of pathogens (McDougald, 2005). Signs of diarrhea, inflammation of different parts of the intestinal tract, or simply typhlitis (inflammation of the wall of the cecum) can be observed as a result of spontaneous infection with *Eimeria* spp. and *Trichomonas gallinarum* (*T. gallinarum*, Mehlhorn, 2016). Spontaneous histomoniasis in poultry leads to damage to the liver and caecum, with the corresponding development of multifocal necrotizing hepatitis and diphtheria typhlitis (Shchebentovska and Holubtsova, 2020). In spontaneous trichomoniasis, there is damage to the oral cavity, pharynx, small and large parts of the intestinal tract, and the formation of granulomas with localization, as a rule, in the liver and cecum (Landman et al., 2019). The development of pathological processes is determined by the relevant biological features of the pathogens, the method of their penetration, and localization in the host's body. It is known that infection of chickens with these pathogens occurs orally (Landman et al., 2019). Since the century-long study of histomoniasis, our understanding of the infection has evolved (Tyzzer, 1919). Initially, it was believed that infection with *Histomonas meleagridis* (*H. meleagridis*) primarily resulted from eating *Histomonas gallinarum* (*H. gallinarum*) eggs, either directly or through earthworms harboring these eggs (Patra et al., 2013). However, recent studies have increasingly reported *H. meleagridis* infections without the presence of nematodes (Badparva and Kheirandish, 2017). Recently, researchers have widely explored alternative modes of chicken infection, specifically highlighting transmission through the mouth and cloaca. Domestic chickens can absorb both liquids and suspended substances through their cloaca, opening a potential avenue for pathogen entry. This mechanism allows pathogens to penetrate the caecum and bursa of Fabricius (Sorvari and Sorvari, 1977; Huber et al., 2006; Patra et al., 2013). This mode of infection has been identified as a risk factor for the dissemination of *H. meleagridis* via the cloaca (Huber et al., 2006, Badparva and Kheirandish, 2017).

When chickens succumb to protozoan infections and these infections have been diagnosed during their lifetime, it becomes crucial to comprehensively study the causes of their death and existing pathological processes. At the same time, additional parasitological studies are of particular importance, contributing to the clear identification and classification of the components of the pathological picture (McDougald and Hu, 2001; Liebhart et al., 2014). For the patho-anatomical diagnosis of parasitic diseases in chickens, it is important to take into account the ways of invasion and the peculiarities of the localization of pathogens in the body. It is especially necessary to carefully differentiate pathological changes in those organs that may be jointly affected by various pathogens. Therefore, during the pathological autopsy, attention should be focused not only on the dominant pattern of changes in organs and tissues but also on the search and differentiation of etiological factors, which is especially relevant in the case of concomitant diseases. At the autopsy of chickens, additional parasitological methods of research can contribute to the establishment of reliable diagnoses, especially in the case of protozoan co-invasions (Liulin et al., 2023). The use of such an approach will allow increasing the reliability of the detection of cases of co-infestation of protozoan dead chickens, in particular, in farms that are unfavorable in terms of eimeriosis, histomonosis, and trichomonosis. This study aimed to investigate and classify macromorphological changes in the intestine, liver, spleen, and bursa of Fabricius during spontaneous co-invasion of *Eimeria* spp., *H. meleagridis*, and *Trichomonas gallinae* in adult domestic chickens.

MATERIALS AND METHODS

Ethical approval

The study was conducted in compliance with the ethical norms and principles of the requirements of the European Union. However, approval of the bioethics commission of the State Biotechnological University (Ukraine) was not required, as the research materials were fresh corpses and samples from adult chickens that died a natural death. Chicken carcasses came from farms located in the Kharkiv region. Their delivery was carried out by the requirements in special thermal containers with ice at a temperature of +4°C within 6 hours after death.

Macromorphological studies

The research was conducted in the period from March 2019 to June 2023. The research material was the carcasses and autopsy substrates of adult chickens aged 1.5-2.3 years that died a natural death, which came from mini-farms in the Kharkiv district of the Kharkiv region. Among them, 24 heads of the Rhode Island breed from a farm where 160 laying hens are kept, and 7 heads of the Adler silver breed from a private farm for 70 laying hens. Chicken carcasses were dissected in the dissection hall of the Department of Normal and Pathological Morphology of the Kharkiv State Zoo Veterinary Academy, Ukraine (since 2021, the State Biotechnological University, Ukraine) by the method of

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evisceration in the dorsal position (Dobin and Kokurichev, 1963). To determine macroscopic changes in organs, depending on their structure, various parameters were determined. During the examination of the liver and spleen, the following were determined: shape, size, color, appearance of the internal structure (in section), consistency, and degree of blood filling. In the intestinal tube and bursa of Fabricius, the state and thickness of the wall, external and internal surfaces, integrity, color, and degree of blood filling were determined; the presence or absence of damage and its degree; the nature of the content (amount, condition, consistency, color). Patency was also determined in the intestinal tube. The condition of the surface, their integrity, color, thickness, presence or absence of lesions, and their nature were determined by examining the skin and serous coverings. The degree of transparency and gloss was also determined on the serous covers. The degree of manifestation of macromorphological changes of organs and tissues found in infested chickens was compared with the results of studies of organs and tissues in the corpses of non-infested chickens (n = 5 Rhode Island breed and n = 3 Adler silver breed of the same age from the indicated farms) that died from various mechanical damage (mainly from limb injuries), and their internal organs and tissues are whole and undamaged.

Coproscopic studies

Samples were used as material for coproscopic studies containing cloaca (feces) and intestinal contents (chyme), which were taken from the corpses of the examined (n = 31) and control (n = 8) chickens. The samples were examined by the native smear method. The procedure involved preparing the sample, which included feces and chyme. These materials were carefully mixed with a 50% water-glycerin solution at a 1:1 ratio in a glass. Then, a drop of the resulting suspension was placed on a slide covered with a cover glass and examined under a microscope (magnification ×80 and ×400, Carl Zeiss, Germany) to detect and identify the pathogens *Eimeria* spp., *H. meleagridis*, and *T. gallinae*. To identify pathogens, scrapings of the mucous membrane of the large intestine (*H. meleagridis* and *T. gallinae*), small intestine (*T. gallinae*), and smears-imprints from the affected organs were examined microscopically. Smears-imprints were fixed with methanol for 3-5 minutes and stained according to the Romanovsky-Giemsa method. To identify trichomonads, smears were air-dried and stained with methylene blue, which made it possible to identify parasites by their morphological features (Menezes et al., 2016).

Flotation method

Eimeria oocysts were detected by the Fülleborn flotation method. For this purpose, fecal samples were obtained from the cloaca of the deceased chickens under investigation. Approximately, 3 g of feces were collected and placed in a glass container. The next step involved introducing a saturated sodium chloride (NaCl) solution into the glass at a ratio of 1:20 while continuously stirring the contents with a glass rod. The resulting suspension was filtered through a metal filter (hole size 0.8-1.0 mm) into similar cups and left for 30 minutes. After settling from the surface of the liquid of the studied samples, 3 drops of the surface film were taken with a metal loop (diameter 0.8 cm), which were transferred to a glass slide and subjected to microscopy for the presence of *Eimeria* oocysts. The intensity of invasion (the number of oocysts in 1 g of feces) was determined by the McMaster method (Vadlejch et al., 2011). Species affiliation of causative agents of *Eimeria* spp. oocysts were determined morphologically by comparing the obtained data (Shape, shell color, presence of micropyle, and other morphological indicators) with the descriptions of the determination tables (Pellerdy, 1974).

Microbiological studies

Samples of pathological material were subjected to bacteriological studies. Smears were stained by Gram and Ziel-Nielsen and examined microscopically for the presence or absence of bacteria (Bortnichuk et al., 2007).

Statistical analysis

Statistical processing of the obtained data was carried out using the MS Excel-2019 application package. Primary statistical processing was performed using the descriptive statistics package. Next input factors were defined: average values of the main feature pathological changes quantity in organs M (in percentage), error of the mean m (in percentage), and the absolute error in determining M - ΔM (in percentage) at a given level of reliability P > 95%, which corresponds to the level of statistical significance $p < 0.05$ according to the Student's criterion (Lebed'ko et al., 2022).

RESULTS

Macromorphological changes in the liver of the studied chickens

According to the results of the autopsy and its analysis, pathological changes in the liver and intestines were diagnosed in all the investigated individuals of the dead chickens (n = 31). Along with the mentioned changes, pathological changes were found in the spleen (n = 15), in the bursa of Fabricius and peritoneum (n = 5), and in the skin (n 3) in the examined chickens. The liver examination revealed a range of different macroscopic lesions in all of the

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chickens studied. These included instances of fatty dystrophy, hemorrhages beneath the liver capsule and within the parenchyma, ruptures of the liver capsule, and hepatomegaly often associated with a combination of steatosis and multifocal necrosis, occasionally forming granulomas. In the majority of chickens, a distinct pattern of liver lesions emerged. This pattern featured necrotic foci in the form of spherical diffuse spots with a central red zone in a light yellowish-gray border. Notably, these necrotic foci were predominantly located in the left lobe of the liver. Additionally, the caudal portions of the organ displayed areas of hemorrhage, with a particularly pronounced presence in the right lobe (Figure 1).

In non-infested chickens, the liver maintained its natural shape and volume. The liver capsule remained undamaged, displaying a moderate level of tension, and the edges of the organ were sharply defined. The color of most of the surface of the liver is uniformly light brown, there were signs of minor venous stasis (Figure 2).

When examining the surface of the liver of infested chickens with the help of optical lenses, surface defects with pits and hemorrhages were detected (Figure 3).

During the examination of the cut surface of the liver (lower right lobe) in 14 individuals of the examined chickens, numerous translucent granulomas (shiny, vitreous, with a white center) were visualized. The presence of these granulomas became especially apparent when viewed under low-power lenses. These granulomas were similar in size to a millet grain, reaching a diameter of up to 0.5 cm (Figure 4).

The degree of damage and the manifestation of macromorphological changes in chickens that died from co-invasion were compared with the morphological indicators of the organs of non-infested (coproscopically pathogens *Eimeria* spp., ***H. meleagridis*** and ***T. gallinae*** were not detected) chickens (n = 8) of the control group. When examining the surface of the section of the liver of non-infested chickens, preservation of the internal structure and uniformly light brown color was observed (Figure 5).

Among the examined chickens, four were diagnosed with hepatomegaly (Figure 6). In these cases, the liver displayed an enlargement characterized by blunted edges and a tense capsule. Some parts of the liver exhibited tears in the capsule. Additionally, differentiation between different sections of the organ was challenging due to these changes. The liver's surface exhibited a varied coloration, featuring alternating areas of orange hue, circular lesions represented by light yellow spots (some of which contained a central red zone), and darker cherry-colored regions indicating hemorrhages. Oily patches resulting from parenchymal tears were also evident, and these areas displayed an increased gloss. Even on a liver section from a dissecting knife blade, an oily coating was apparent. The consistency of the liver was notably flaccid.

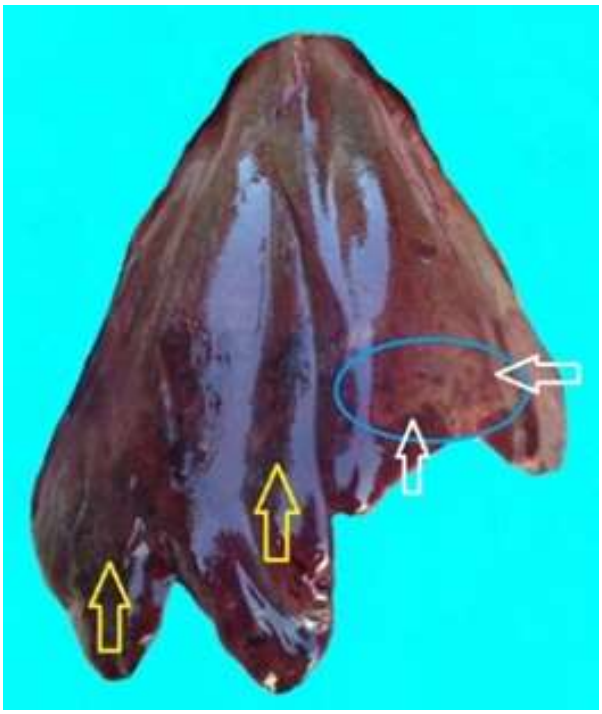

Figure 1. Macroscopic lesions of the liver of a Rhode Island breed chicken aged 24 months with fatal co-invasion of *eimeriosis*/histomonosis/trichomonosis, lesions in the form of spherical diffuse spots with a central red zone in a light yellowish-gray border (white arrows in a blue oval), areas of hemorrhages (yellow arrows; Kharkiv region, Ukraine, 2023).

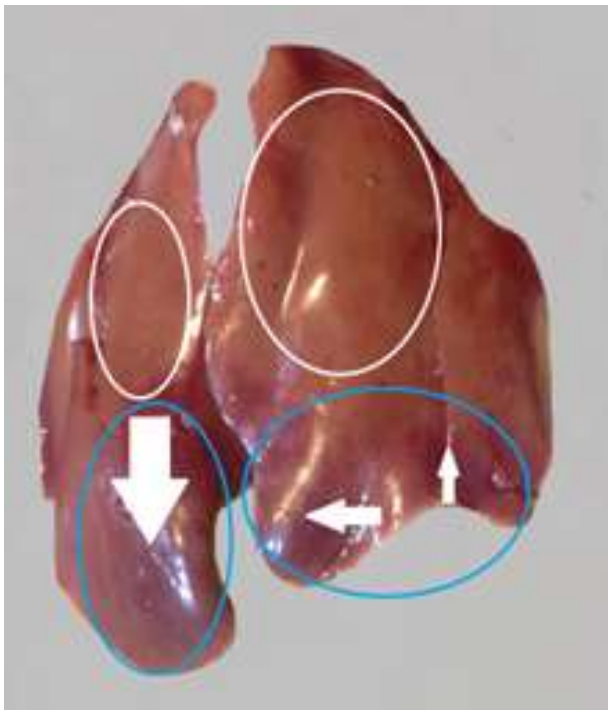

Figure 2. Liver of a non-infested Rhode Island breed chicken aged 24 months with uniform light brown coloration of the surface, white ovals, and slight venous congestion of the caudal areas (white arrows in blue ovals; Kharkiv region, Ukraine, 2023).

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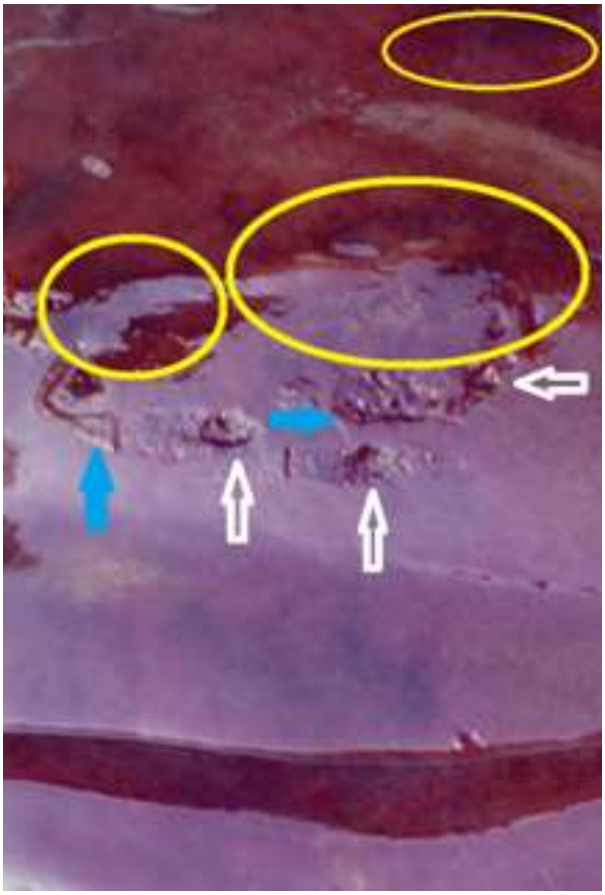

Figure 3. The surface of the liver of a dead Rhode Island breed chicken aged 24 months with fatal co-invasion of eimeriosis/histomoniasis/trichomoniasis: rupture of the capsule (blue arrow); defects in the form of dimples on the surface of the organ (white arrows); areas with hemorrhages (yellow ovals, magnification x5; Kharkiv region, Ukraine, 2023).

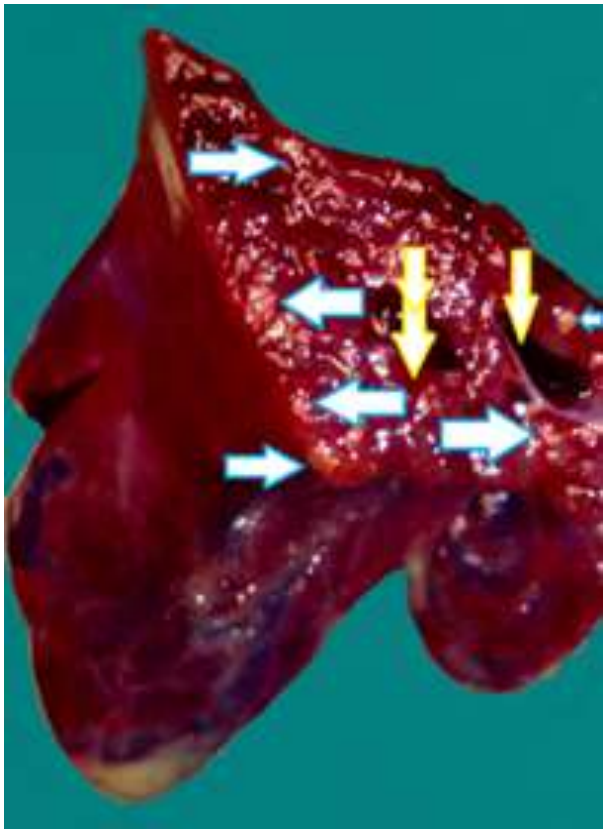

Figure 4. Fragment of a section of the caudal edge of the right lobe of the liver of a Rhode Island chicken aged 24 months with co-invasion of eimeriosis/histomoniasis/trichomoniasis (whitish translucent granulomas on the surface of the liver section [horizontal arrows]; dilated left vessels [vertical arrows], magnification X3, Kharkiv region, Ukraine, 2023).

Figure 5. Fragment of the caudal edge of the right lobe of the liver of a non-infested Rhode Island breed chicken aged 24 months (uniform light brown coloring of the surface of the liver section; Kharkiv region, Ukraine, 2023).

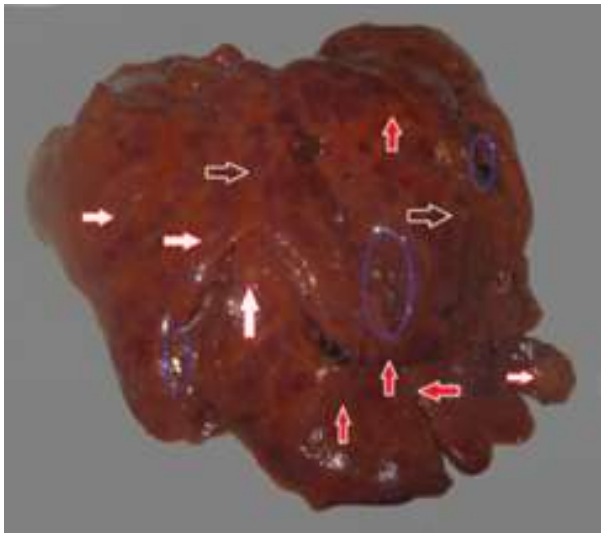

Figure 6. Hepatomegaly in a domestic Rhode Island breed chicken aged 27 months due to co-invasion with eimeriosis/histomonosis/trichomonosis (light-yellow necrosis foci [arrows]; areas of parenchymal rupture with increased gloss due to steatosis, ovals, Kharkiv region, Ukraine, 2020).

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Macromorphological changes of the intestine in the studied chickens

Various changes in the wall and mesentery of the intestinal tube were observed in all the examined chickens. Mucocatarrhal inflammation of various parts of the intestine was observed in most chickens (diagnosed duodeno-jejuno-ileo-typhoproctitis). The severity of the lesion was dominated by changes in the cecum and its diverticula. The cecum was markedly enlarged, with a widened lumen and a partially thickened (with some exceptions) wall (Figure 7). Whereas in non-infested chickens, the cecums had a moderate size and preserved integrity and wall thickness. In the apical parts, there was a slight expansion of the lumen (Figure 8). In the majority of chickens examined in the period 2019-2020, signs of hyperplasia, small hemorrhages, and swelling of the mucous membrane were observed in the locations of the diverticula of the cecum. Deformation of the wall of the cecum and its mesentery was observed in five chickens due to the presence of numerous granulomas with uneven thickening and expansion of their lumen (Figures 9, 10). In three chicken specimens, the examination of the intestinal tube revealed a significant distortion caused by the presence of numerous granulomas on the serous membrane. These granulomas were spherical or ellipsoid in shape, characterized by a translucent and vitreous appearance, with a distinctive white center. They were dense in texture and varied in size from as small as a poppy seed to 0.7 cm in diameter (Figure 11). In non-infested chickens, the serous membrane of the intestinal wall was smooth, moderately spasmodic, intact, and uniformly colored in a light pink color (Figure 12). In three chickens, masses of mucus and dark brown-red detritus were found in the lumen of the distal part of the small intestine (Figure 13). Comparatively, the mucous membrane of the jejunum was intact in non-infested chickens, uniformly colored in a light pink color, and moderately shiny (Figure 14).

Figure 7. Caecum of an Adler silver laying hen age 2 years with co-invasion with eimeriosis/histomoniasis/trichomoniasis: bulbous expansion of the lumen due to the content of inflammatory exudate, masses of detritus and gas bubbles (Kharkiv region, Ukraine, 2020).

Figure 8. Caecum of a non-infested Adler silver laying hen aged 24 months: Preservation of the body shape of the intestines, green ovals; slight expansion of their tops (blue arrows; Kharkiv region, Ukraine, 2020).

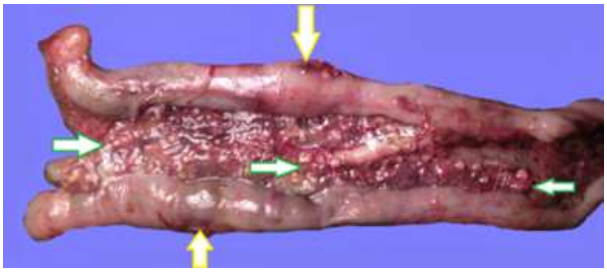

Figure 9. Caecum of a Rhode Island breed chicken age 24 months with co-invasion with eimeriosis/histomonosis/trichomonosis. deformation, thickening of the wall, and expansion of the lumen, vertical arrows. Numerous granulomas in the mesentery, (horizontal arrows; Kharkiv region, Ukraine, 2023).

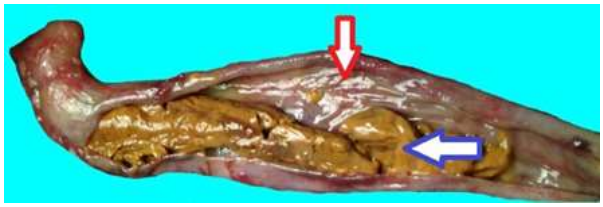

Figure 10. Caecum and its contents in a Rhode Island breed chicken (aged 24 months) due to co-invasion with eimeriosis/histomonosis/trichomonosis. Thickening of the wall (vertical arrow); plasticine-like clumps of mustard color in the lumen due to copro stasis (horizontal arrow; Kharkiv region, Ukraine, 2023).

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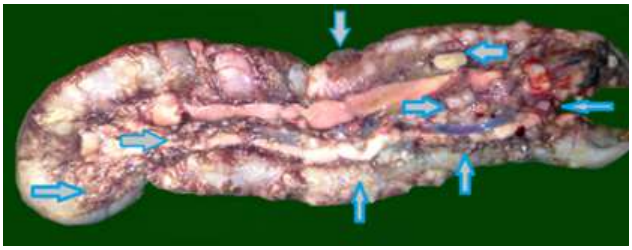

Figure 11. The U-shaped knee of the duodenum with mesentery and pancreas of a chicken (aged 21 months, Rhode Island breed) with co-invasion with eimeriosis/histomoniasis/trichomoniasis (numerous granulomas on the mesentery and the serous membrane of the intestinal wall (arrows; Kharkiv region, Ukraine, 2023).

Figure 12. U-shaped knee of the duodenum with mesentery and pancreas of a non-infested Rhode Island breed chicken (aged 21 months) smooth surface of the serous membrane of the intestinal wall, no deformations, uniform coloring (Kharkiv region, Ukraine, 2023).

Figure 13. Fragment of the distal part of the dissected jejunum of an Adler silver chicken aged 20 months with co-invasion of eimeriosis/histomoniasis/trichomoniasis (Mucous masse [blue arrows]; brownish-red necrotic masses in the lumen [yellow arrow], Kharkiv region, Ukraine, 2019).

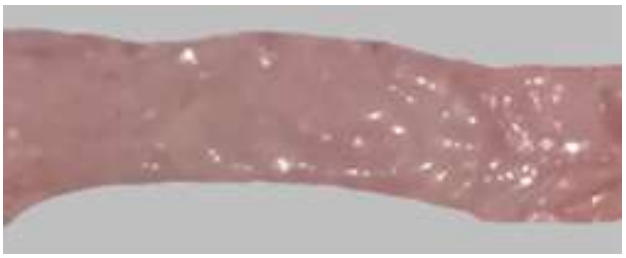

Figure 14. Fragment of the distal part of the dissected jejunum of a non-infested Adler silver chicken aged 20 months integrity, light pink color, and moderate gloss of the mucous membrane (Kharkiv region, Ukraine, 2019).

Macromorphological changes of the spleen in the studied chickens

The spleen of 15 investigated chickens was partially changed in shape, characterized by the presence of small whitish-yellow nodules on its surface. The spleen also displayed a slight increase in volume. On cross-section, the spleen's coloration appeared uneven, featuring whitish-yellow nodules and beige-gray areas against a backdrop of cherry coloring. Additionally, structureless zones of necrosis were evident (Figure 15). The spleen exhibited preserved macroscopic characteristics, including its shape, volume, color, and internal structure (Figure 16).

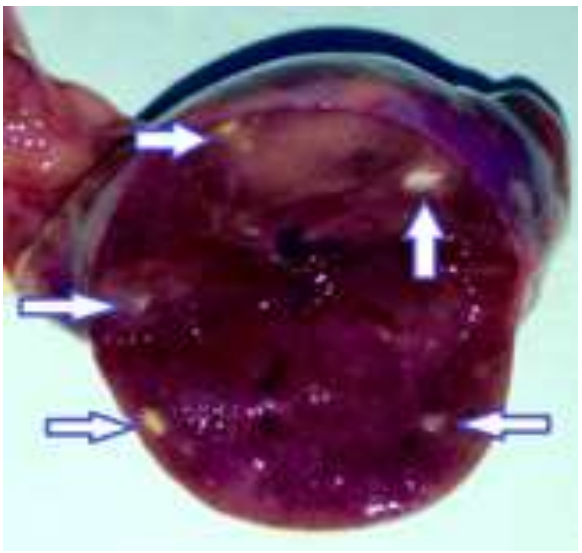

Figure 15. View of the cut surface of the spleen of a Rhode Island chicken aged 18 months that died from co-invasion with eimeriosis/histomoniasis/trichomoniasis. Whitish-yellow granulomas in the thickness of the organ, arrows (Kharkiv region, Ukraine, 2021).

Figure 16. Surface view of a section of the spleen of a non-infested Rhode Island chicken aged 18 months preserved structure and uniform coloring of the surface and section (Kharkiv region, Ukraine, 2021.

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Macromorphological changes of the bursa of Fabricius in the studied chickens

The bursa of Fabricius in five examined chickens indicated an ovoid shape and an increased volume (approximately 3-4 times compared to the normal). It was in the form of a large cyst, the walls of which contained granulomas (Figure 17). In non-infested chickens, the bursa of Fabricius had a preserved spherical-ellipsoidal shape and a normal volume (Figure 18).

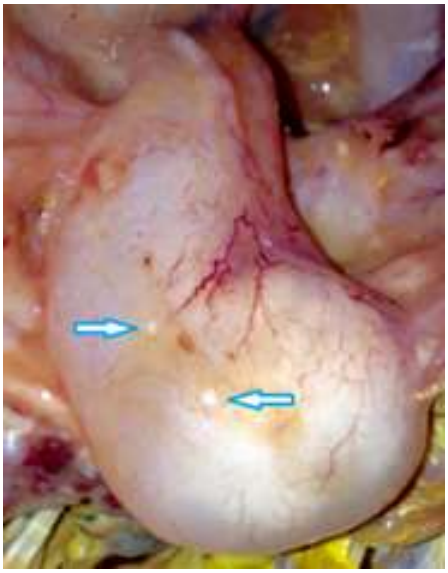

Figure 17. Bursa of Fabricius of a Rhode Island chicken aged 18 months with co-invasion with with eimeriosis/histomonosis/trichomonosis. Ovoid shape, increased volume, granulomas in the wall (arrows; Kharkiv region, Ukraine, 2023).

Figure 18. General view of the bursa of Fabricius of a non-infested Rhode Island chicken aged 18 months preserved volume and shape (Kharkiv region, Ukraine, 2023).

Macromorphological changes of the peritoneum in the studied chickens

During the study of the serous coverings of the thoracic-abdominal cavity in some chickens, there were nodular formations of various sizes with localization, in particular, on the peritoneum (Figure 19).

Macromorphological changes in skin changes in the studied chickens

In three of the examined chickens, upon examining the inner part of the skin, accumulations resembling white-colored translucent and shiny bead-like granulomas were found (Figure 20). The presence of identified patho-anatomical changes in the studied chickens was confirmed by coproscopic examinations of the intestinal tube contents from chicken carcasses. The detection of pathogens included *Eimeria* spp. with an invasion intensity ranging from 78.9 ± 5.2 to 103.5 ± 7.6 oocysts per 1 gram of feces. Furthermore, *H. meleagridis* was identified in the cecum and necrotic liver areas. *Trichomonas gallinae* was found in the oral cavity and pharynx, mucosal structures of the intestinal wall, and necrotic areas (granulomas) of the liver, as well as the mesentery, bursa of Fabricius, and oviduct. The frequency of lesions and manifestations of pathological changes in co-invasion are indicated in Table 1.

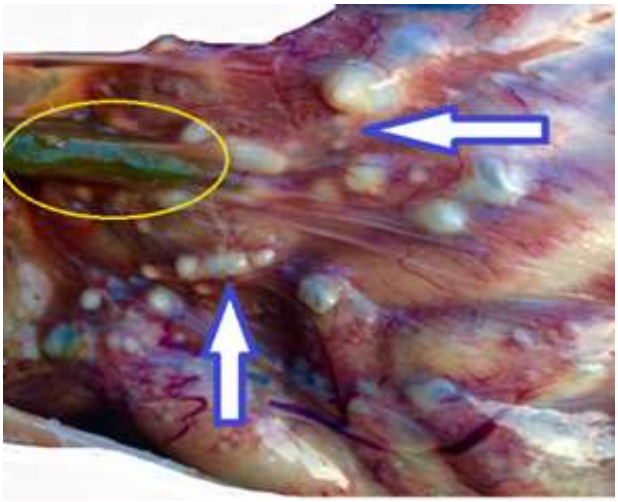

Figure 19. Granulomas on the peritoneum of a Rhode Island chicken aged 24 months due to a fatal co-invasion with Eimeriosis/histomonosis/trichomonosis (arrows). Oval: gall bladder (Kharkiv region, Ukraine, 2023).

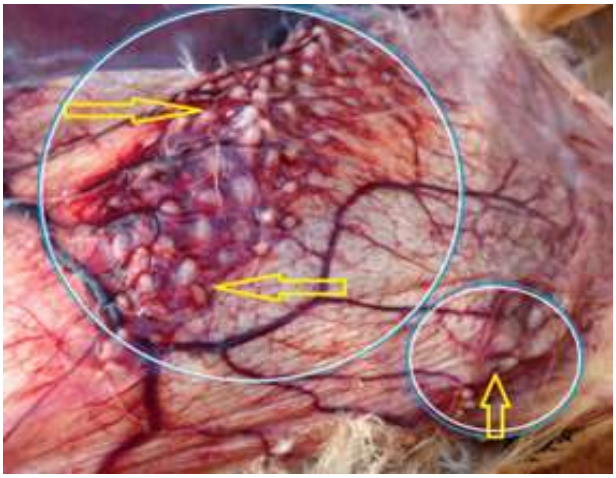

Figure 20. Granulomatous lesions of the skin (arrows in ovals) of a domestic Rhode Island chicken aged 24 months due to co-invasion with eimeriosis, histomonosis, and trichomoniasis (Kharkiv region, Ukraine, 2023).

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Table 1. Localization of macroscopic lesions of the organs and tissues after co-invasion of eimeriosis, histomoniasis, and trichomoniasis in laying hens (n = 31)

Number of chickens/year of the study	Liver	Intestine	Spleen	Bursa of Fabricius	Peritoneum	Skin
1-4/2018	+	+				
5-8/2019	+	+				
9/2020	+	+				
10-12/2020	+	+	+			
13-14/2020	+	+				
15-16/2021	+	+	+			
17/2021	+	+				
18/2021	+	+	+			
19/2021	+	+				
20-24/2021	+	+	+			
25-27/2023	+	+				
28-29/2023	+	+	+	+	+	+
30/2023	+	+	+	+	+	
31/2023	+	+	+	+	+	+
TOTAL	31	31	15	5	5	3
Percentages (or probability)	100	100	48.39	16.13	16.13	9.68
M	0	0	9.12	6.72	6.72	5.34
P	0	0	0.05	0.05	0.05	0.05
Δ*M*	0	0	18.63	13.71	13.71	11.02

M: Sample mean, m: Error of the mean, p: Confidence level (this is the probability that the given value can be equal to zero), ΔM: The absolute measurement error at a confidence level of $p < 0.05$.

As can be seen in Table 1, all examined deceased chickens (n = 31) exhibited diagnosed changes in both the liver and intestines, accounting for 100% occurrence. Additionally, 15 chickens displayed alterations in the spleen in conjunction with the liver and intestinal lesions, constituting 48.39 ± 9.12% ($p < 0.05$). Furthermore, in 5 chickens, the liver and intestine lesions were combined with lesions of the bursa of Fabricius, representing 16.13 ± 6.72% ($p < 0.05$), and the peritoneum, also at 16.13 ± 6.72% ($p < 0.05$). Among three chickens, concurrent damage to internal organs coincided with localized skin damage, accounting for 9.68 ± 5.34% ($p < 0.05$).

DISCUSSION

According to the obtained results, indications of duodenal jejuno ileotyphlitism were identified in the intestines of the examined chickens. This diagnosis was substantiated by the presence of *Eimeria* spp. pathogens, manifesting in varying oocyst counts ranging from 78.9 ± 5.2 to 103.5 ± 7.6 per 1 gram of feces. The specific species detected encompassed.

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) for a total number of oocysts, accounting for 19.2%, 7.3%, 15.5%, 5.2%, 9.3%, 3.2%, and 40.3%, respectively ($p < 0.05$). This distribution aligns with findings reported by Yatusevich et al. (2016) as they have their species-specific localization in the intestines. The damage inflicted by *Eimeria* spp. on the surface layers of the mucous membrane, leading to exudative processes involving lymphoid structures and exhibiting manifestations of hyperplasia, lends support to pathological anatomical changes in the intestines of pigeons with experimental eimeriosis (Liulin et al., 2021) and data reported by Kot et al. (2020), where eimeriosis serous-catarrhal, catarrhal-mucous-hemorrhagic enteritis and necrosis of the intestinal mucosa were detected in quails. Furthermore, parallels in these changes are evident in chickens subjected to experimental infection with *Eimeria maxima*, *Eimeria tenella*, *Eimeria necatrix*, and *Eimeria acervulina*. These alterations, encompassing hemorrhagic typhlitis and acute catarrhal or catarrhal-hemorrhagic enteritis, were documented by Hirkovy (2016). During experimental eimeriosis in turkeys, Liulin (1994) noted the development of desquamative-proliferative enteritis, thereby reinforcing the concurrence of these findings.

In an experimental invasion of turkeys, simultaneous exposure to both *T. gallinarum* and *Eimeria adenoides* highlighted the influence of *Eimeria* on the development of pathological changes associated with trichomoniasis. This influence extended to the cecums, affecting their condition and manifesting through the absence of yellow foamy liquid in stools. When *Eimeria* was absent, trichomoniasis mainly affects one of the cecums (Norton, 1997).

Within the examined chickens' caeca, characteristic lesions indicative of histomonosis were identified, including thickening of their walls and hyperemia. In 16.13 ± 6.72% ($p < 0.05$) of chickens, deformation of the wall of the cecum

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and its mesentery was detected due to the presence of numerous granulomas with uneven thickening and expansion of their lumen. Multiple foci of limited necrotic areas, varying in size, were found in the liver of the examined chickens. Similar changes in the liver of chickens, marked by distinctive hepatomegaly and the presence of ulcerative lesions on the surface, displaying round depressions and raised peripheral edges, which had a unique crater-like shape, namely, necrosis and chronic inflammation with a classic manifestation of the "apple" type (dark red centers and pale outer rims) are supported by data (Ficken, 2020).

Distinctive aspects of liver damage during the co-invasion of chickens included the specific localization of necrotic foci primarily within the left lobe of the liver. Additionally, areas of hemorrhage were prevalent in the caudal fragments, particularly prominent in the right and larger lobes of the liver. Combined liver damage with characteristic hepatomegaly against the background of a combination of steatosis, multifocal necrosis, and granulomas (shiny, vitreous, with a white center) was established, which was microscopically confirmed by the presence of *H. meleagridis* and *T. gallinae* pathogens. The findings reported by Burns et al. (2013), as well as the data obtained in the study of pelicans, have revealed the presence of multifocal necrotizing hepatitis and splenitis. his corresponds with observations made in studies on histomonosis manifestations in both turkeys (Abd El-Wahab et al., 2021), chickens (Liebhart et al., 2017) and typhlohepatitis of chickens caused by *T. gallinae* (Hauck et al., 2019; Landman et al., 2019). Granulomatous pathological changes in unusual areas of the bursa of Fabricius in the studied chickens were caused by the lysis of its lymphocytes. This process resulted in the depletion of bursal follicles and cystic degeneration of the organ and led to the formation of a large thin-walled cyst with granulomas in its wall. This pattern of changes resonates with the findings of Karaman et al. (2009) for histomonosis in turkeys. Pathological changes in the bursa of Fabricius contribute to systemic damage to the body, and accordingly, to the death of chickens. The systemic effect of co-invasion pathogens (*Eimeria* spp., *Histomonas meleagridis,* and *Trichomonas gallinae*) is confirmed by the presence of granulomatous lesions of the peritoneum and skin and is consistent with the results of studies obtained (Sentíes-Cué et al., 2009).

In 48.39 ± 9.12% ($p < 0.05$) of the affected chickens, pathological changes of the spleen were observed with the presence of small nodules and areas of necrosis on its surface and the section. Similar lesions of the spleen, pale or yellow foci, were also found by Sentíes-Cué et al. (2009) for histomonosis of turkeys. Such lesions of the spleen naturally lead to lymphoid exhaustion and the development of an immunosuppressive state and immunodeficiency, which is confirmed by Kim et al. (2019). First of all, the immunosuppressive effect on the body of chickens is carried out by *Eimeria* spp. which contributes to the development of co-invasion of histomoniasis and trichomoniasis (Popp et al., 2011). The detection of pathological anatomical changes in the spleen and other organs and the development of immunodeficiency during eimeriosis, histomoniasis, and trichomoniasis are also indicated (Singh et al., 2008).

The indicated data and features of patho-anatomical changes have applied diagnostic value. However, the presence of the pathogen *H. meleagridis* in these organs may not cause inflammation and pathological changes for a long time (Lotfi et al., 2014), which depends on the state of the immune system and species characteristics. For these reasons, chickens are considered partially resistant to histomonosis although the disease often remains hidden. Such chickens remain carriers and a reservoir of pathogens for a long time, thereby contributing to the spread of *H. meleagridis* and creating certain difficulties in diagnosis (Beer et al., 2022). Manifestations of pathological changes enterohepatic and the degree of granulomatous lesions of internal organs in co-infested chickens (intestine, liver [100%], spleen [48.39%], bursa of Fabricius [16.13%]) correspond to the results of studies obtained by Karaman et al., (2009) and Landman et al. (2019) in histomonosis and trichomonosis of chickens.

It is known that the diagnosis of co-invasion is confirmed by the detection of specific pathogens, but the presence of the pathogen *H. meleagridis* and/or *T. gallinae* in the caecum without the manifestation of specific pathological changes is not considered to be an indicator of morbidity (Powell et al., 2009). It has been experimentally established that trichomonads can stay in the cecum for 7 months or longer without inducing clinical manifestations or causing the mortality of chickens (Powell et al., 2009; Zahoor et al., 2011). As a result, trichomonads can frequently exist among chickens on poultry farms, often without being clinically detectable (Dolka et al., 2015). It was reported that there were no macroscopic pathological changes in dead finches and that the European epidemic strain of *T. gallinae* was isolated from them (Zu Ermgassen et al., 2016). It is known that birds kept in captivity, compared to free-living ones, are more often sick with eimeriosis, histomonosis, and trichomonosis (Tuska-Szalay et al., 2022). The microscopically detected presence of *T. gallinae* is quite often the cause of the death of wild sparrows (Doyle et al., 2022). At the same time, there is a relationship between pathogen isolates from sparrows and pathogens isolated from chickens, which was proven by molecular studies (Amin et al., 2014). The pathological changes found in the examined chickens as a result of co-infestation are also confirmed by studies conducted in the USA which revealed necrotizing typhlitis and hepatitis in peacocks (*Pavo* spp.) caused by the pathogens *H. meleagridis* and *Tetratrichomonas gallinarum* (Clarke et al., 2017). The causative agents of *H. meleagridis* and *T. gallinarum* have been isolated and identified in peacocks indicating characteristics consistent with histomonosis (Clarke et al., 2017). However, the role of the latter in pathogenesis remains unclear and requires further research. The intensive reproduction of *Eimeria*, *histomonads*, and *trichomonads* takes place within the mucous membrane structures of the intestinal wall, leading to inflammation and necrosis. The presence of the

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specified lesions and the pathogens detected at the same time is confirmed by Liebhart et al. (2014) and McDougald and Hu (2001), who believe that the severity of *H. meleagridis* liver damage can increase the presence of *Eimeria*, particularly *Eimeria tenella*. Thus, the revealed macromorphological changes in co-invasion of eimeriosis/histomonosis/trichomonosis supplement previously obtained data and expand the possibilities of postmortem diagnosis.

CONCLUSION

The obtained results showed that macroscopic lesions of liver, intestines, spleen, bursa of Fabricius, peritoneum, and skin were found in the examined chickens. This variant of the lesions is systemic and indicated multiple organ failure and a expressed the immunodeficiency. Further histopathological studies are needed to study the microscopic changes in the organs and tissues of domestic chickens during co-infestation.

DECLARATIONS

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Authors' contributions

Petro Lyulin conducted a coproscopy and bacteriological study and described their results. Lyubov Lyakhovych conducted the pathological examination, anatomical dissection, and photography, and described and analyzed the data. Mykola Bogach identified and described pathogens. Olena Byrka took part in patho-anatomical dissections and anatomical dissection. Alla Petrenko assisted in the patho-anatomical autopsies. 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

The authors declare no conflict of interest.

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.

Availability of data and materials

All data from the current study are available by request from the authors.

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