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# MICROSCOPIC CHANGES IN LAYING HENS KIDNEYS AT THE EGG DROP SYNDROME

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***Abstract.*** *The article presents the results of studying the microscopic changes in laying hens' kidneys under egg drop syndrome (EDS-76). Clinical signs of EDS-76 in laying hens included decreasing of egg production, suppression, changes in egg pigmentation, laying of small-sized eggs with a soft shell, or without a shell at all. During the dissection enlarged, pale kidneys with clearly dilated blood vessels were registered. The lungs in most cases had an uneven red-pink color. In many laying hens, the spleen had an uneven color – it showed areas of different sizes and shapes of grayish, bluish, and red. In some cases, the liver was unevenly colored (with areas of yellowish and clay color), and in other cases, point and spotted hemorrhages were found under its capsule. However, the most pronounced and permanent gross changes registered in reproductive organs. During the histological examinations of the kidneys of laying hens infected with EDS-76 virus, we found that in this organ, there are specific for this disease and nonspecific microscopic changes, which are registered under the kidney damage of various etiologies. Specific for EDS-76 changes include the presence of basophilic and eosinophilic inclusion bodies in the nuclei of many epithelial cells in convoluted tubules, part of epithelial cells of straight tubules, and part of endothelial cells, podocytes, and mesangiocytes of the many glomeruli. Some of nuclei completely acquired distinctly oxyphilic properties. Nonspecific microscopic changes included diverse changes in nuclei and cytoplasm in epithelial cells of convoluted and straight tubules, violations of intercellular junctions, damages of renal corpuscles.*

***Keywords:*** *laying hens, egg drop syndrome, clinical signs, gross changes, kidneys, microscopic changes.*

## *Introduction*

Broiler meat and chicken eggs are cheap foods. Feed costs for protein and energy production in poultry are the lowest compared to other livestock industries. The production of 1 g of pure protein in eggs and meat or poultry feed units is 8 times less than in beef, 3 times less than in pork. It takes 12 times less time to produce 1 ton of meat than in cattle breeding, and 8 times less than in pig breeding (Vermienko, 2008; Nazarenko, 2009). One egg satisfies a human daily need for vitamin B2 by 10–12%, vitamin D – by 10–40%, vitamin A – by 15–16% (Oshchipok, 2007).

Various infectious and non-infectious diseases cause great problems for poultry (Giasuddin et al., 2002). Infectious diseases mainly cause decreased egg production in hens. These diseases include infectious bronchitis, Newcastle disease, infectious encephalomyelitis, avian pox, 4th serotype of chicken adenovirus group I and the egg drop syndrome (Badar et al., 2006; Geetha et al., 2008). The egg drop syndrome (EDS-76) is primarily a significant economic problem due to product shortages and additional costs for diagnostic and prevention (Begum et al., 2013).

EDS-76 is an infectious viral disease in laying hens, characterized by a sharp decrease in egg production, damage of the reproductive organs, changes in pigmentation and egg shape, thinning and deformation of the shell, reduced protein value, reduced percentage of hatching chicks from hatching eggs and reduced viability of chicks (Anufrieva & Olshevskaya, 1992; McNulty & Smyth, 2002).

In addition to chickens, EDS-76 registered in quail, pheasants, and guinea fowl. Turkeys can be infected experimentally, but they do not show clinical signs (Parsons et al., 1980). Antibodies to EDS-76 virus were found in a large number of bird species: domestic and wild ducks and geese, herons, seagulls, owls, storks and swans and pigeons (Schloer, 1980; Watanabe & Ohmi, 1983; Das & Pradhan, 1992; Bidin et al., 2007; Ezema et al., 2010).

Until 2001, it was thought that EDS-76 virus was not virulent to ducks and geese. However, in 2001, this virus was isolated at the outbreak of the respiratory disease in young goslings, and the disease was reproduced experimentally (Ivanics et al., 2001).

In many countries, EDS-76 virus infects up to 50 % of chicken flocks (Badar et al., 2006). Chickens of any age and all breeds and crosses are susceptible to this infection (Zsak & Bartha, 1979; Al-Hilly et al., 1982; Curtis & Boachie, 1982; Howell, 1982; Ibrahimov & Osidze, 1988; McFerran & Smyth, 2000; Elayo et al., 2010).

Infection can occur at any age, but the clinical signs of the disease are limited to the period of egg production, which is probably due to the reactivation of the latent virus (McFerran et al., 1978; Kaleta et al., 2003; Suresh et al., 2013).

Pathomorphological changes under EDS-76 are only superficially described in only a small number of original researches (Bakulin et al., 1988; Van Eck et al., 1976; Taniguchi et al., 1981; Brugh et al., 1984; Chetty et al., 1987; Ivanics et al., 2001).

According to McFerran and Smyth (2000), pathognomonic changes in laying hens are short-lived and are present only during the period of falling egg production.

According to many authors, pathomorphological changes are generally minimal and limited by the reproductive tract of laying hens. Inactive ovaries,

oviduct atrophy, edema, and white exudate in the uterus may be registered. In the uterus – dystrophic changes and desquamation of epithelial cells, glandular atrophy, infiltration by heterophiles, lymphocytes, and plasma cells. In the epithelial cells of the uterus, cervix, and vaginal area – intranuclear inclusion bodies (Taniguchi et al., 1981; Brugh et al., 1984; Chetty et al., 1987).

Bakulin et al. (1988) in addition to changes in the reproductive tract of laying hens infected by the EDS-76 virus, found enlarged kidneys, numerous hemorrhages under the liver capsule, atrophy of the cloacal bursa and spleen, and the accumulation of exudate in the pericardium and intramuscular connective tissue. EDS-76 virus is also minimally replicate in the gastrointestinal tract (McFerran, 2000; Smyth, 2000).

## *Materials and methods of research*

The research was performed at the poultry farm "Khorostpodillya" of the Khmelnitskyi region on laying hens of "Hisex White" and "Hisex Brown" crosses. The diagnosis on EDS-76 was established by epidemiological data, clinical signs, and results of the dissection and laboratory researches. Laboratory researches (ELISA-test) was performed in the Center for Veterinary Diagnostics (Kyiv).

Dissection of 28 laying hens (11 laying hens of the cross "Hisex White" and 17 laying hens of the cross "Hisex Brown") was performed by the method of partial evisceration in the conventional sequence (Zone et al., 2009). During the dissection, the pieces from different parts of the kidneys (at least 3 pieces from each kidney) were selected for histological examination

Pieces of the kidney were fixed in 10% aqueous formalin solution with a pH 7.2–7.4, dehydrated in ethanol of increasing concentration, and through chloroform embedded into paraffin. Slides with a thickness of $8 \pm 1$ μm were stained with hematoxylin and eosin (Goralsky et al., 2005). All steps were carried out at room temperature. The slides were studied under a light microscope Micros 100LED (Austria) at magnifications of x 50–1500. Photographing of slides was performed through a photographic nozzle NDPL-2(2X) using a camera Canon EOS 550D.

## *Results of the research and their discussion*

Clinical signs of EDS-76 in laying hens included decreasing of egg production, suppression, changes in egg pigmentation, laying of small-sized eggs with a soft shell, or without a shell at all.

During the dissection of laying hens with EDS-76 registered enlarged, pale kidneys with clearly dilated blood vessels. The lungs in most cases had an uneven red-pink color. In many laying hens, the spleen had an uneven color – it showed areas of different sizes and shapes of grayish, bluish, and red. In some cases, the liver was unevenly colored (with areas of yellowish and clay color), and in other cases, point and spotted hemorrhages were found under its capsule.

However, the most pronounced and permanent changes registered in reproductive organs. In some laying hens, gross changes in the ovaries and oviduct were not detected, except for hyperemia in individual oocytes at the early stages of development.

In most laying hens, the redness of the vast majority, or all oocytes, and inflammation of the oviduct was marked. In the

lumen of the caudal part of the oviduct were often formed eggs with a soft shell, which easily burst at low pressure on it. The protein of such eggs was turbid.

In some laying hens, egg formation completely stopped in the early stages. This was evidenced by the complete absence of oocytes in the late stages of formation and the absence of formed eggs in the caudal part of the oviduct. In some laying hens, signs of egg maturation and egg formation were not detected at all. The tendency to reduce the weight of the whole oviduct or its individual parts was detected in laying hens with EDS-76.

In addition to these signs, in some laying hens was the serous catarrh of the small intestine, as well as atrophy of the cloacal bursa and spleen.

During the histological examinations of the kidneys of laying hens infected with EDS-76 virus, we found that in this organ, there are specific for this disease and nonspecific microscopic changes, which are registered under the kidney damage of various etiologies.

Specific for EDS-76 changes include the presence of basophilic and eosinophilic inclusion bodies in the nuclei of many epithelial cells in convoluted tubules, part of epithelial cells of straight tubules, and part of endothelial cells, podocytes, and mesangiocytes of many glomeruli (Fig. 1–3). Some of the nuclei completely acquired distinctly oxyphilic properties (Fig. 1).

Previously, in a cell culture model found that virus particles and four types of virus-induced inclusions appeared in the nucleus in 24 hours after infection (Adair et al., 1979). The dynamics of their development is similar to that of avian adenoviruses of subgroup I (Mar-

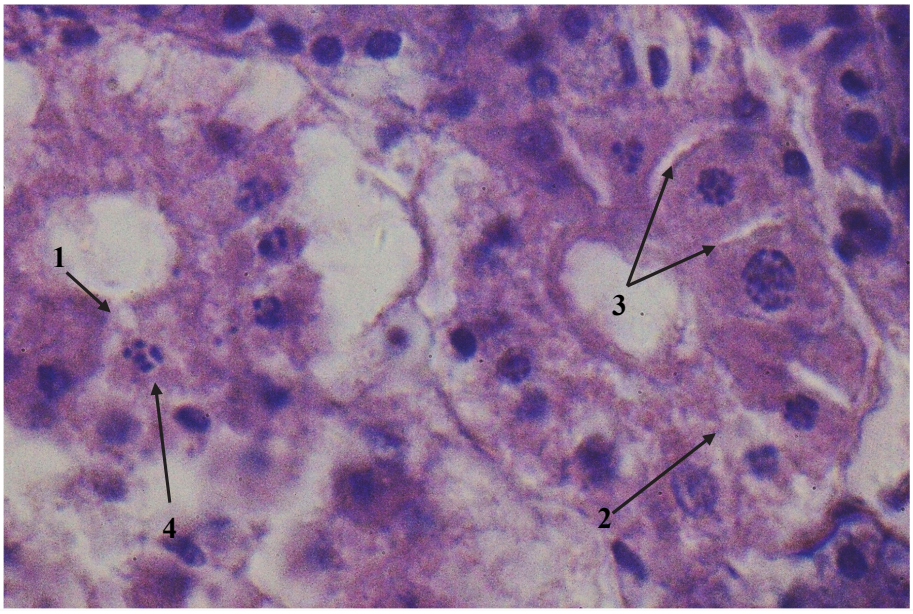


**Fig. 1. Convoluted tubules in the kidney of a laying hen under EDS-76:** 1 – lysis of the apical cytoplasm of the epithelial cell; 2 – lysis of all parts in the cytoplasm of the epithelial cell; 3 – discomplexation of neighboring epithelial cells; 4 – basophilic inclusion bodies in the eosinophilic nucleus of the epithelial cell. Hematoxylin and eosin, x 1000

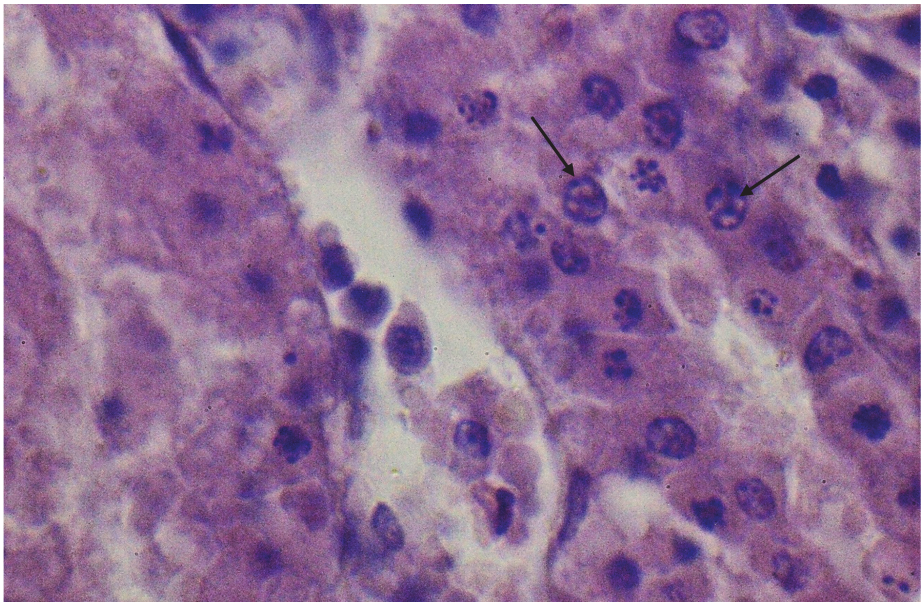

**Fig. 2. Convoluted tubules in the kidney of a laying hen under EDS-76: eosinophilic inclusion bodies in the nuclei of epithelial cells (arrows). Hematoxylin and eosin, x 1000**

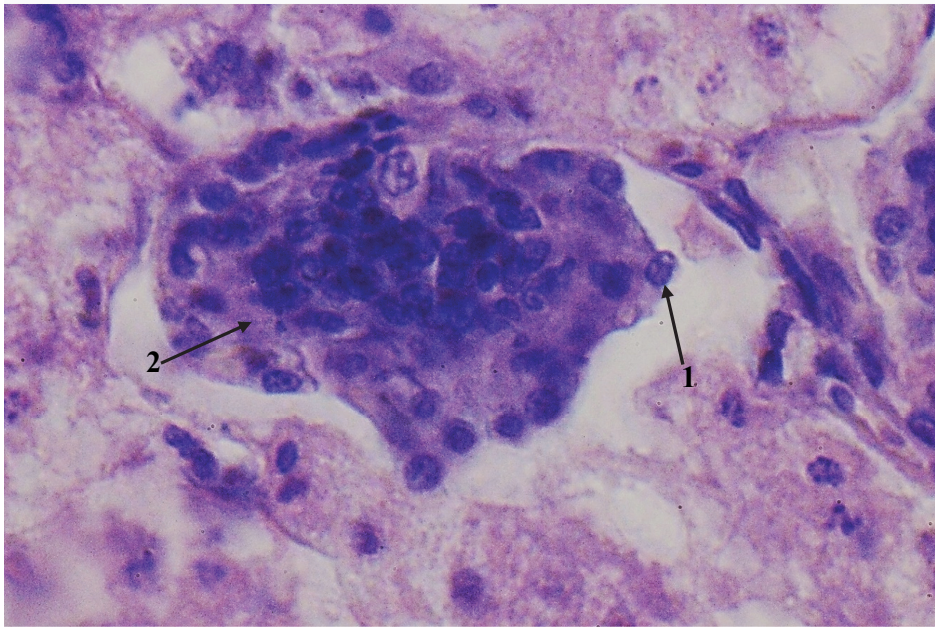


**Fig. 3. Renal body of a laying hen under EDS-76:** 1 – eosinophilic inclusion bodies in a nucleus of a mesangiocyte; 2 – eosinophilic substance. Hematoxylin and eosin, x 1000

tinez-Palomo et al., 1967; Adair et al., 1978; Adair et al., 1979).

Under EDS-76 the intranuclear inclusion bodies are found in epithelial cells of the uterus, a cervix uterus, and a vaginal site (Taniguchi et al., 1981; Brugh et al., 1984; Chetty et al., 1987).

Staining of monolayer cells with hematoxylin and eosin can be used to control EDS-76 virus replication in cell culture. The first changes are detected after 24 hours. They manifest as several eosinophilic inclusion bodies in the nuclei of infected cells. Later, one or two large inclusion bodies were found in the nucleus, which consists of separate clusters. The nucleoplasm around the inclusion bodies acquires a granular structure, separates from the nuclear envelope and nucleolus, and forms one or more basophilic pockets, separated by unpainted areas (Adair et al., 1979).

It was also found, that kidney and liver trypsinized cell cultures can be used to isolate EDS-76 virus. The virus reproduces the best in duck kidney cell culture, worse – in duck and chicken liver cell cultures, and very poorly – in turkey kidney and liver cell cultures (Adair et al., 1979).

Considering the literature, we believe that we found that intranuclear inclusion bodies in various kidney cells reflect replication of EDS-76 virus in them. In the convoluted tubules, basophilic inclusion bodies were found in nuclei of many epithelial cells. The number of such inclusion bodies in nuclei of epithelial cells was different – from 4 to 14.

The size of inclusion bodies was small – from 0.5 to 2.7 μm. The shape of intranuclear basophilic inclusion bodies was also different. Most of them

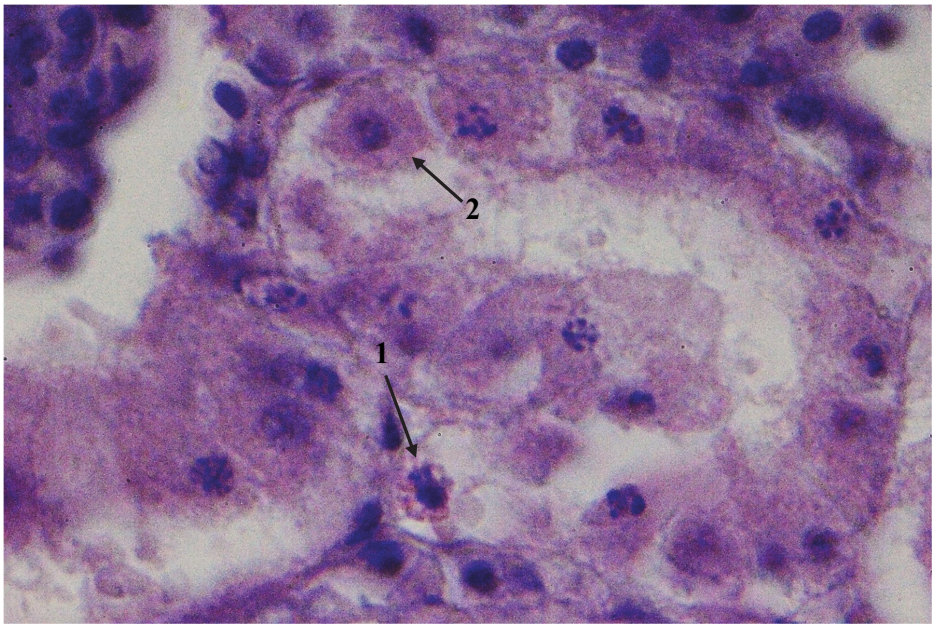


**Fig. 4. Convoluted tubules in the kidney of a laying hen under EDS-76:**
1 – basophilic inclusion bodies in the cytoplasm of the epithelial cell; 2 – round-shaped epithelial cell. Hematoxylin and eosin, x 1000

were round or oval in shape, but in some nuclei, there were inclusion bodies of elongated and irregular shapes.

In some epithelial cells, basophilic inclusion bodies were found in the cytoplasm (Fig. 4). With the destruction of epithelial cells, basophilic inclusion bodies remained among the cell detritus, and with complete lysis of the cells, they lay freely in the lumen of the tubule.Basophilic inclusion bodies from the chromatin of the nucleus were well-differentiated based on the following features. First, the inclusion bodies were much more intensely stained in blue color with hematoxylin compared to chromatin. Second, inclusion bodies remained basophilic even when all other parts of the nucleus acquired oxyphilic properties. Third, basophilic inclusion bodies remained intact even after the complete lysis of epithelial cells.

In convoluted tubules were also found non-specific for EDS-76 changes. Epithelial cells of all convoluted tubules were in a state of cell swelling. As a result, their size significantly increased. In the part of the convoluted tubules, the increase in the volume of epithelial cells was so significant that the lumen of the tubule completely or almost completely disappeared (Fig. 5).

In some epithelial cells, the cytoplasm lysis was observed. Cytoplasmic lysis is registered in different areas of epithelial cells: basal, apical, and perinuclear (Fig. 1, 5). However, most of the cytoplasm in many cases was lysed in the basal part of the cells.

Complete or partial discomplexation of adjacent altered epithelial cells of convoluted tubules was detected in some places (Fig. 1). At the same time, part of the discomplexed epitheliocytes

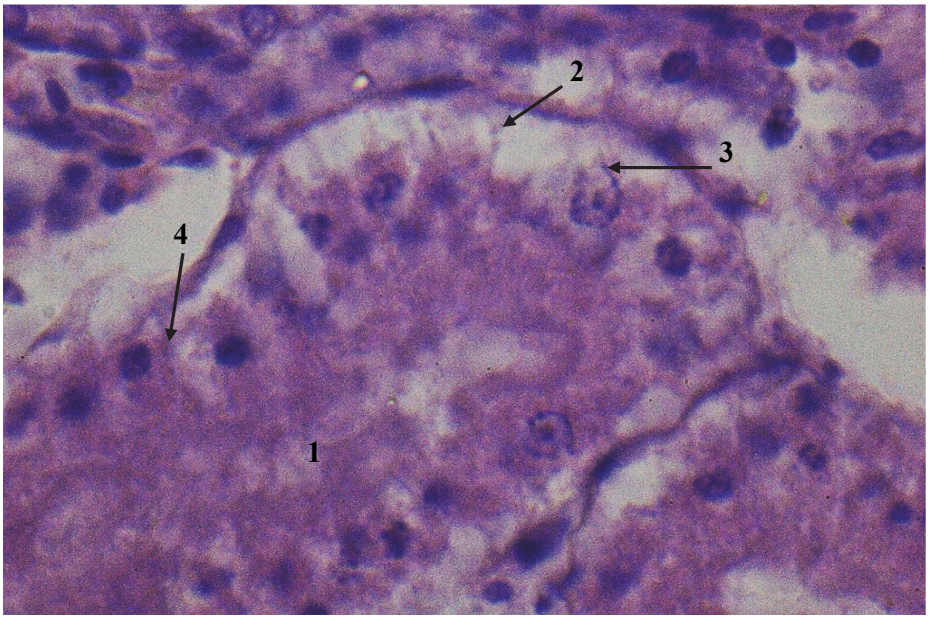


**Fig. 5. Convoluted tubules in the kidney of a laying hen under EDS-76:**
1 – lack of tubular lumen; 2 – lysis of the basal part of cytoplasm in the epithelial cell; 3 – lysis of the nuclear envelope in the epithelial cell; 4 – cell swelling. Hematoxylin and eosin, x 1000

acquired a rounded shape (Fig. 4). Part of the epithelial cells was separated from the basal membrane into the lumen of the tubule.

In many tubules, there is pronounced subepithelial edema, as a result of which all epithelial cells were separated by a continuous layer from the basal membrane (Fig. 6).

In a part of the epithelial cells of the convoluted tubules, changes in shape, increase in the size of the nucleus, and the presence of protrusions of the nuclear envelope were registered. Partial lysis of the nuclear envelope and nucleus of epithelial cells was also observed (Fig. 5).

In the straight tubules in the kidneys of laying hens with EDS-76, the microscopic changes were slightly different from similar changes in the convoluted tubules. Signs of cell swelling and partial lysis of the cytoplasm were registered in their epithelial cells. In many areas, partial or complete discomplexation of neighboring epithelial cells and their complete or partial separation from the basal membrane were detected.

Hyperchromatosis of the nuclear envelope was registered in the nuclei of some cells. Part of the epithelial cells of the straight tubules were destroyed. In some cells, karyorrhexis was detected in some cases. Intranuclear inclusion bodies in straight tubules were found only in individual epitheliocytes.

Many renal corpuscles had irregular shapes. In glomeruli registered proliferation of mesangiocytes. Sometimes registered a significant expansion of the exit of the proximal convoluted tubule from the renal corpuscle (Fig. 6).

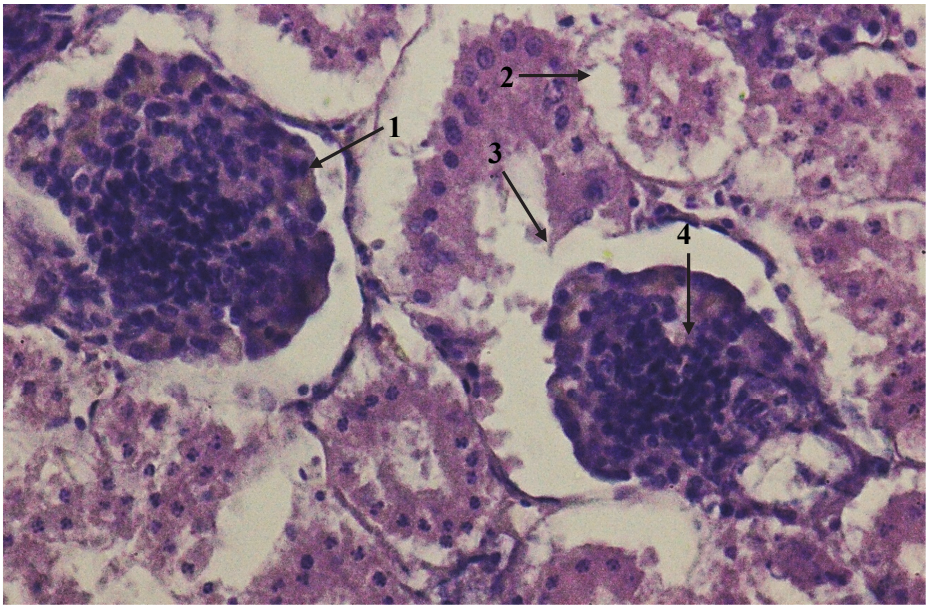


**Fig. 6. The kidney of a laying hen under EDS-76:** 1 – renal corpuscle of irregular shape; 2 – subepithelial edema; 3 – significant expansion of the exit of the proximal convoluted tubule from the renal corpuscle; 4 – proliferation of mesangiocytes. Hematoxylin and eosin, x 200

## *Conclusion*

In the kidneys of laying hens under EDS-76 were registered specific and nonspecific changes. Specific changes should be considered in the pathomorphological diagnosis of EDS-76.

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***Б. В. Борисевич, В. В. Лісова (2020). МІКРОСКОПІЧНІ ЗМІНИ В НИРКАХ КУРЕЙ-НЕСУЧОК ПРИ СИНДРОМІ ЗНИЖЕННЯ НЕСУЧОСТІ.*** *Ukrainian Journal of Veterinary Sciences, 11(3): 46–55, https://doi.org/10.31548/ujvs2020.03.005*

***Анотація.*** *У статті представлені результати дослідження мікроскопічних змін нирок курей-несучок за синдрому зниження несучості (СЗН). Клінічні ознаки СЗН у курей-несучок включали зниження несучості, придушення, зміни пігментації яєць, знесення яєць невеликих розмірів з м'якою оболонкою або взагалі без шкаралупи. Під час розтину реєструються збільшені бліді нирки з чітко розширеними кровоносними судинами. Легені здебільшого мали нерівномірний червоно-рожевий колір. У багатьох курей-несучок селезінка мала нерівномірний колір – у ній виявлялися ділянки різних розмірів і форми сіруватого, синюшного та червоного кольору. У деяких випадках печінка була нерівномірно забарвлена (з ділянками жовтуватого та глинистого кольору), а в інших випадках під її капсулою були виявлені точкові та плямисті крововиливи. Однак, найбільш виразні та постійні грубі зміни реєструвались у репродуктивних органах. Під час гістологічних досліджень нирок курей-несучок, інфікованих вірусом СЗН, ми виявили, що в цьому органі є специфічні для даного захворювання та неспецифічні для нього мікроскопічні зміни, які реєструються при ураженні нирок різної етіології. Специфічні для СЗН зміни включали наявність базофільних та еозинофільних тілець-включень у ядрах багатьох епітеліальних клітин звивистих канальців, частини епітеліальних клітин прямих канальців та частини ендотеліальних клітин, подоцитів та мезангіоцитів багатьох клубочків. Деякі ядра повністю набули виразно оксифільних властивостей. Неспецифічні мікроскопічні зміни включали різноманітні зміни ядер та цитоплазми епітеліальних клітин звивистих та прямих канальців, порушення міжклітинних з'єднань, пошкодження ниркових тілець.*

***Ключові слова:*** *кури-несучки, синдром зниження несучості, клінічні ознаки, макроскопічні зміни, нирки, мікроскопічні зміни*