ISSN: 1220-3173; E-ISSN: 2457-7618

MORPHOPATOLOGICAL RESEARCHES IN THE RESPIRATORY FORM OF INFECTIOUS AVIAN BRONCHITIS AT PIGEONS

CERCETĂRI MORFOPATOLOGICE ÎN FORMA RESPIRATORIE A BRONȘITEI INFECȚIOASE AVIARE LA PORUMBEI

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ABSTRACT | REZUMAT

The researches were carried out between March 2017 and April 2019, by performing necropsies on 37 corpses of pigeons, both male and female, of various breeds especially of house pigeon (*Columba livia f. domestica*) at the Forensics and Necropsy Diagnosis Department of the Faculty of Veterinary Medicine in Timisoara, in order to clarify the cause of death. Of these, 13 cases showed predominantly lesions of the respiratory tract, located in the trachea, lungs and air sacs. The bodies were necropsied using the technique specific to this species. After evisceration, samples were collected (2/1.5 cm tissue fragments), following a detailed macroscopic examination of the organs, from: larynx, trachea, lung and air sacs. The samples were fixed in 10% formaldehyde solution for 24 hours, processed through the paraffin method. The obtained blocks were sectioned using a microtome, at 6 µm, stained using the trichrome method - Haematoxylin - Eosin - Methylene blue (HEA) for overall examination and the Giemsa method for cellular details. Avian infectious bronchitis (IB), the respiratory form, was diagnosed morphopathologically at 13 cases (35.13%) out of 37 pigeon corpses examined necropsically and histopathologically. From the cases studied, the following were identified: a) specific and/or characteristic lesions: serous, haemorrhagic and/or fibrinous tracheitis; congestion, oedema and catarrhal bronchopneumonia; serous and/or fibrinous aerosaculitis; uric serositis; b) non-specific lesions: cardiac and hepatic dystrophies; lymphomonocytic myocarditis; lymphomonocytic meningoencephalitis. The recommendations following this study are related to: ensuring the optimal microclimate, feeding and hygienic-sanitary conditions for this species; implementation of prophylaxis and control measures specific to pigeons; avoiding the contact and common habitability of different bird species.

Keywords: pigeon, *Coronaviridae*, infectious bronchitis, bronchopneumonia

Cercetările au fost efectuate în perioada martie 2017-aprilie 2019, la Disciplina de Medicină legală și Diagnostic necropsic, din cadrul Facultății de Medicină Veterinară din Timișoara, prin necropsierea a 37 de cadavre de porumbei, sex F și M, de diferite rase, cu precădere porumbelul de casă (*Columba livia f. domestica*), în vederea elucidării cauzei morții. Dintre acestea, 13 cazuri au prezentat leziuni predominant ale aparatului respirator, cantonate în trahee, pulmoni și sacii aerieni. Cadavrele au fost necropsiate prin tehnica specifică acestei specii. După eviscerare s-a efectuat examinarea macroscopică amănunțită a organelor luate în studiu: laringe, trahee, pulmon și a sacilor aerieni, ulterior au fost prelevate probe pentru examen histopatologic (fragmente de țesuturi de 2/1,5 cm). Probele au fost fixate în soluție de formaldehidă 10% timp de 24 de ore, prelucrate prin metoda "la parafină". Blocurile obținute au fost secționate la microtom, la 6 µm, colorate prin metoda tricromică hematoxilină-eozină-albastru de metil (HEA) pentru examinare de ansamblu și metoda Giemsa pentru detalii celulare. Bronșita infecțioasă aviară, forma respiratorie, a fost diagnosticată morfopatologic la 13 cazuri (35,13%) din 37 cadavre de porumbel examinate necropsic și histopatologic. La cazurile luate în studiu au fost identificate următoarele: a) leziuni specifice și/sau caracteristice: traheite seroase, hemoragice și/sau fibrinoase; congestie, edem și bronhopneumonie catarală; aerosaculită seroasă și/sau fibrinoasă; serozită urică; b) leziuni nespecifice: distrofii cardiace și hepatice; miocardită limfomonocitară; meningoencefalită limfomonocitară. Recomandările în urma acestui studiu țin de: asigurarea condițiilor de microclimat, furajare și igienico-sanitare optime pentru această specie; realizarea măsurilor de profilaxie și combatere specifice porumbeilor; evitarea contactului și habitabilității comune a diferitelor specii de păsări.

Cuvinte cheie: porumbel, *Coronaviridae*, bronșită infecțioasă, bronhopneumonie

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The causative agent of Avian Infectious Bronchitis is the BIA virus (Infectious Bronchitis Virus - IBV) belonging to the *Coronaviridae* family, genus *Coronavirus*.

The viral particle has dimensions of 80 - 120 nm, and the genome consists of RNA. The virus has tropism for the epithelial cells of the anterior respiratory tract, for those of the oviduct, and for the epithelium of the urinary tract (1, 2, 5, 6, 11, 13, 17).

The importance of the disease lies in the high mortality, in the slowdown of the growth rate and the poor conversion of the fodder, in pigeons, the Massachusetts serotype has a similarity of almost 100% with the isolated / obtained strains from chickens (4, 9, 10).

The most important lesions are in the respiratory system, especially in the trachea, while the functional and histopathological changes of other tissues are reduced or even absent (13, 17).

A significant number of studies suggest that non-Galliform birds are affected by IBV and may play an important role in AvCoV transmission and ecology (4, 8). It has been shown that transmission is not only from pigeons to pigeons or between pigeons and other bird species, but also from pigeons to humans, quite common in breeders who will end up with bird fancier's lung (BFL) which is one of the most common types of is the most common form of hypersensitivity pneumonitis characterized especially through pneumonia with centrilobular fibrosis (3, 12, 15). Understanding the diversity of genotypes, the study of specific and non-specific morphopathological lesions, the eco-epidemiology of the disease in different environments, is very useful and necessary to establish good prophylaxis and control. (9, 10, 13, 14).

MATERIALS AND METHODS

The researches were carried out between March 2017 and April 2019, by performing necropsies on 37 corpses of pigeons, both male and female, of various breeds especially of house pigeon (*Columba livia f. domestica*) at the Forensics and Necropsy Diagnosis Department of the Faculty of Veterinary Medicine in Timisoara, in order to clarify the cause of death. Of these, 13 cases showed predominantly lesions of the respiratory tract, located in the trachea, lungs and air sacs. The bodies were necropsied using the technique specific to this species (7, 16). After evisceration, samples were collected (2/1.5 cm tissue fragments), following a detailed macroscopic examination of the organs, from: larynx, trachea, lung and air sacs.

The samples were fixed in 10% formaldehyde solution for 24 hours, they were modelled and reintroduced into a new formaldehyde bath for final fixation for two days. The fixed samples were passed into the dehydration battery, consisting of alcohol in increasing concentrations, from 70 degrees to absolute alcohol. In each of the 5 baths, the samples were kept for 2 hours, after which the alcohol was removed by introduction into benzene to clarify the section, then the paraffin bath was placed in a thermostat at 56 °C. By paraffining, blocks were obtained containing samples (fragments) of injured organs, which were sectioned at the microtome at 6 µm. The obtained sections were fixed on well-degreased slides with Mayer albumin. The sections glued to the slides were stained by the Masson trichromic method (H.E.A.) modified by V. Ciurea with methyl blue for overall examination and the Giemsa method for cellular details (16). The histopathological preparations thus obtained were examined with an optical microscope (Olympus CX41) with increasing objectives, the most eloquent tissue lesions were interpreted and then microphotographed.

RESULTS AND DISCUSSIONS

At the external examination of the corpses, in most cases was noticed the state of advanced cachexia with the highlighting of the sternal hull (Fig. 1). The eyes were clogged in the orbits (enophthalmia), the conjunctival mucosa was pale and the nasal and buccal mucosa were covered by a grayish-whitish, sticky deposit (catarrhal exudate) - coryza and catarrhal stomatitis. Internal examination. In most cases, fine, granular, white-pearly-uric serositis deposits were present on the thoraco-abdominal serosa. The air sacs on 11 corpses were opaque and covered with a network of gray-yellow, friable, partially scaly fibrin, which in some places blurred transparency - fibrinous aerosaculitis.

Fig. 1. Pigeon corpse: granular, white-pearly, fine deposits - uric serositis

Fig. 2. Catarrhal-haemorrhagic tracheitis: the mucosa was covered with a thin layer of reddish mucus

The trachea in most corpses, macroscopically, the mucosa was covered by a thin layer of reddish mucus, which after removal allowed its visualization while maintaining the red colour even after washing - haemorrhagic tracheitis (Fig. 2).

Microscopically, the following were observed: swelling of the ciliated pseudostratified columnar epithelium, hyperplasia of the ciliated cells accompanied by decylation and falling in the mass of sero-catarrhal exudation - catarrhal tracheitis; haemorrhagic exudate at the boundary between the hyaline cartilage and the fundamental substance (Fig. 3).

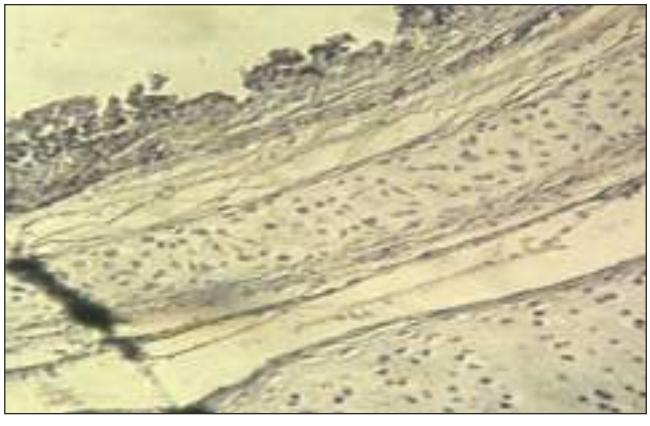

Fig. 3. Catarrhal-haemorrhagic tracheitis: hyperplasia of ciliated cells, decillation and falling in the mass of sero-catarrhal exudation; haemorrhagic exudate at the boundary between the hyaline cartilage and the fundamental substance. Col. HEAx20

The lungs, in eight cases, macroscopically, were enlarged in volume, glossy, bright red, with evidence of costal fingerprints and negative docimasia - active pulmonary congestion (Fig. 4).

Fig. 4. Pigeon corpse - active pulmonary congestion

Microscopically, were identified: ectasia of the alveolar capillaries with the presence in the alveolar septa of intensely bright red coloured erythrocytes, which causes thickening of the alveolar septa and implicitly the reduction of the alveolar lumen and of the respiratory space - active pulmonary congestion (Fig. 5).

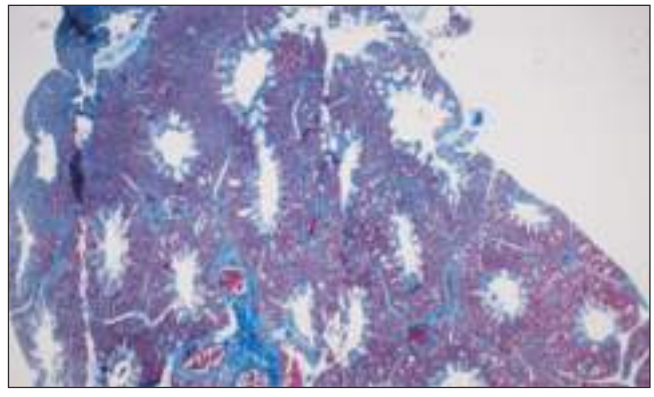

Fig. 5. Active pulmonary congestion: ectasia of the alveolar capillaries with the presence in the alveolar septa of bright red intensely coloured erythrocytes. Col. HEAx10

In five cases, the lungs were: enlarged in volume, of light red colour on inspection and on a section, with a moist, glossy and pasty appearance; at digital pressure on the section surface a reddish, foamy liquid was expressed; hydrostatic docimasia was between waters - inflammatory pulmonary oedema (Fig. 6).

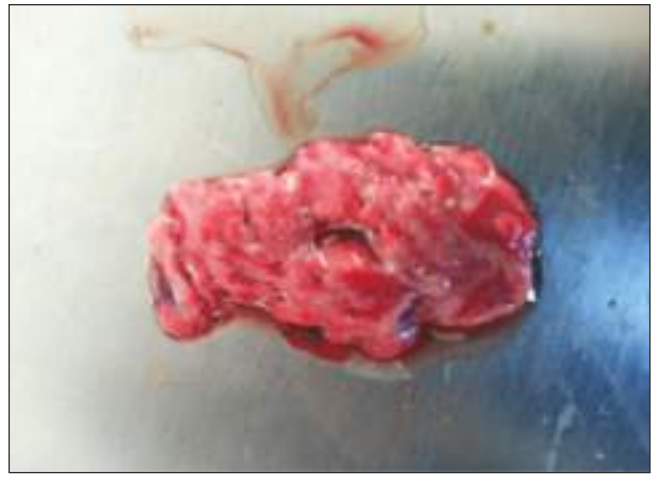

Fig. 6. Inflammatory pulmonary oedema: light red, moist, glossy, pasty appearance; reddish, foamy liquid was expressed on the section surface

The lungs with inflammatory oedema, on microscopic examination had revealed in the lumen of the alveoli and parabronchi an exudation with either basophilic or oxyphilic tint that enlarge and produce hypotrophy by compression of the septa and parabronchial walls. The oedema fluid occupies the respiratory space and consequently causes hypoxia and implicitly respiratory failure - inflammatory pulmonary oedema (Fig. 7).

In seven cases, macroscopically the lungs were: enlarged in volume, red-cherry colour on inspection and on

section; increased consistency and on the section at digital pressure a whitish-gray creamy fluid was expressed: fragments collected from lung tissue inserted into a vessel with water fall to the bottom of the vessel (positive docimasia) - catarrhal bronchopneumonia (Fig. 8).

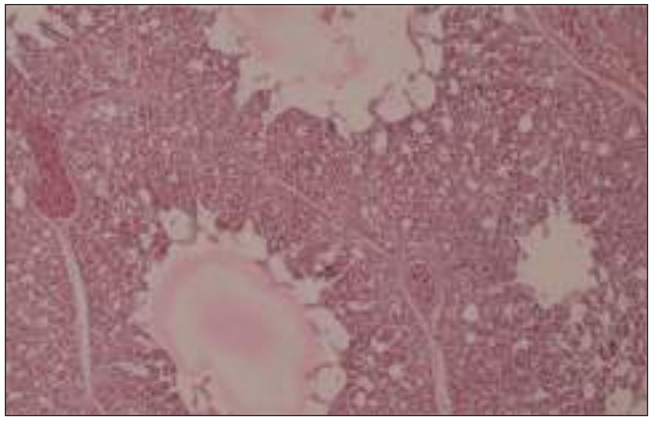

Fig. 7. Inflammatory pulmonary oedema: inflammatory exudate (oxyphilic mass) in the lumen of the parabronchia. Col. HEAx20

Fig. 8. Catarrhal bronchopneumonia: increase in volume, red-cherry colour on inspection and on section, increased consistency

Fig. 9. Catarrhal bronchopneumonia: intraparabronchial catarrh. Col. HEAx40

Microscopically, the following were visible: ectasia of the alveolar capillaries; intricate serous exudation with desquamated pneumocytes in the pulmonary alveolar lumen (intraalveolar catarrh), swelling, partial detachment and falling of the parabronchial epithelium into the lumen, in the mass of serous exudation (intraparabronchial catarrh) - catarrhal bronchopneumonia (Fig. 9). The heart, the liver, macroscopically were increased in volume and weight of gray-yellow colour, without gloss, with the appearance of boiled meat look and friable consistency - myocardosis (Fig. 10) and protein hepatosis.

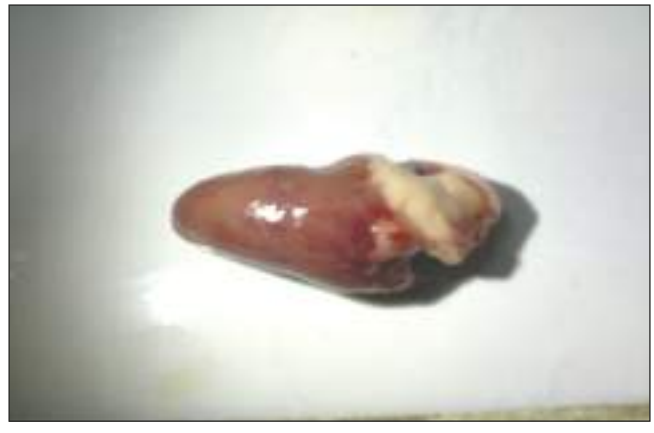

Fig. 10. Heart, increased in volume and weight of gray-yellow color - protidic myocardosis

Microscopically, upon examination with x10, x20, x40 objectives, in the cytoplasm of myocardocytes and hepatocytes were observed basophilic granulations of different sizes (megamitochondria) that give the dark appearance of the cytoplasm, hence the name cloudy intumescence attributed to granular dystrophy - granular myocardosis (Fig. 11).

Megamitochondrias are due to the excess accumulation of serum albumin and serum globulins in mitochondria, which in light microscopy are highlighted by basophilic granules that give the intumescence appearance of cytoplasm.

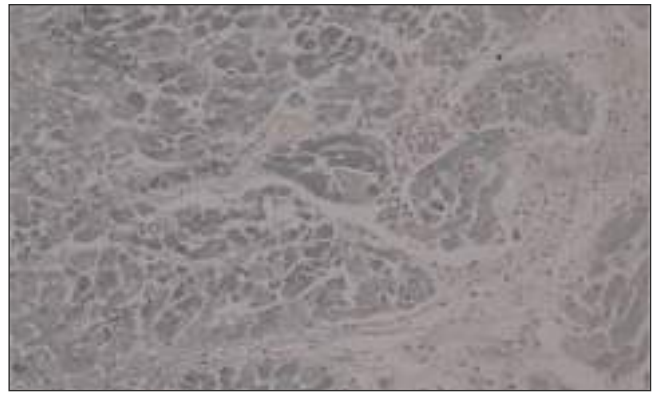

Fig. 11. Myocardium, cross section - protidic - granular myocardosis: basophilic granulations in the cytoplasm, cloudy intumescence. Col. HEAx20

In 14 microscopic cases, lesions of lymphohistiocytic myocarditis and lymphomonocyte meningoencephalitis were identified.

Microscopically, lymphohistiocytic myocarditis is expressed by ectasia of myocardial capillaries, interstitial interfibrilar lymphohistiocytic hyperplasia, but with interfascicular predominance, which causes atrophies, dystrophies and necrosis of myocardial fibers - lymphohistiocytic myocarditis (Fig. 12).

Fig. 12. Lymphohistiocytic myocarditis: lymphohistiocytic hyperplasia accompanied by dissociation, hypotrophy and fragmentation of myocardial fibers. Col. HEAx10

Lymphohistiocytic myocarditis together with the mentioned pneumopathies determines in time the cardio-respiratory insufficiency, responsible for the serious prognosis, lethal in the cases with infectious avian bronchitis.

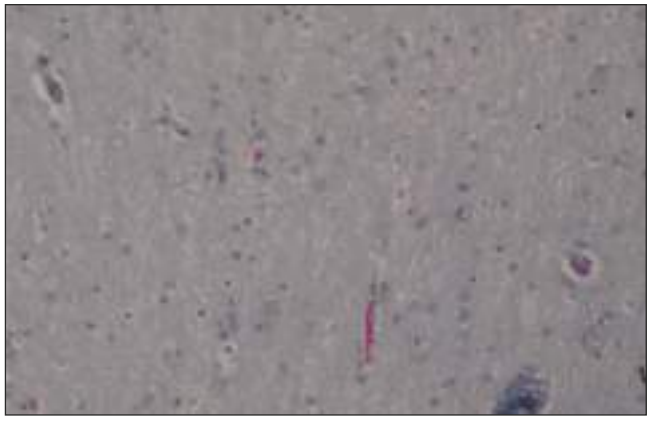

Fig. 13. Lymphomonocytic meningoencephalitis: congestions, oedema, neurodystrophies, perineuronal oedema and monocytic perivascularitis. Col. HEA x 10

Lymphomonocytic meningoencephalitis reported, in eight cases, only microscopically because the macroscopic lesions are inconclusive for establishing the morphopathological diagnosis of encephalitis, being externalized by congestion and meningeal oedema.

Microscopically, with magnifications x10 it is highlighted in the nervous mass: congestions, oedema, neurodystrophies, perineuronal oedema and monocytic perivascularitis (Fig. 13); with targets x20, x40, perivascular, intramural lymphomonocyte and glial nodules are observed. Limphomonocytic inflammation of encephalus, morphologically, suggests the viral origin of the infection.

The following recommendations should be applied:

- Ensuring the optimal microclimate, ensuring feeding and optimum hygienic-sanitary conditions for this species;
- Implementation of prophylaxis and control measures specific to pigeons;
- Avoidance of contact and common habitability of different species of birds, as most recent studies show that strains isolated / obtained from pigeons are 95-100% similar to those from chickens and /or other species, which plays an important role in AvCoV transmission and ecology (4, 9, 13, 15).

CONCLUSIONS

The respiratory form of avian infectious bronchitis was diagnosed morpho-pathologically at 13 cases (35.13%) out of 37 pigeon corpses examined necropsically and histopathologically.

From the cases studied, the following specific and / or characteristic lesions were identified: serous, haemorrhagic and / or fibrinous tracheitis; congestion, oedema and catarrhal bronchopneumonia; serous and / or fibrinous aerosaculitis; uric serositis.

The non-specific lesions were cardiac and hepatic dystrophies; lymphomonocytic myocarditis; and lymphomonocytic meningoencephalitis.

REFERENCES

1. *Barr D.A., Reece R.L., O'Rourke D., Button C., Faragher J.T.,* (1988), Isolation of infectious bronchitis virus from a flock of racing pigeons. Aust Vet J, 65(7):228
2. *Bărăităreanu S.,* (2020), Coronavirus infections: a brief review, Revista Romana de Medicina Veterinara, 30(1):71-76
3. *Bourke S., Anderson K., Lynch P.,* (1989), Chronic simple bronchitis in pigeon fanciers. Relationship of cough with expectoration to avian exposure and pigeon breeders' disease. *Chest*, 95(3):598-601
4. Cavanagh D., (2005), Coronaviruses in poultry and other birds. Avian Pathol, 34:439-448
5. *Cavanagh D.,* (2007), Coronavirus avian infectious bronchitis virus. Vet Res, 38(2):281-297
6. *Cătana N., Herman V., Petrec Oana, Iancu Ionica,*

(2014), Identification of strains belonging to QX pathotype in an outbreak of avian infectious bronchitis, Lucr şt Med Vet, IAŞI, 57(3-4):203-205

7. *Cătoi C.,* (2003), Necropsy diagnosis (in Romanian), (Ed.) Academic Press, Cluj-Napoca, Romania
8. *Chen G.Q., Zhuang Q.Y., Wang K.C.,* (2013), Identification and survey of a novel avian coronavirus in ducks. *PLoS One*, 8(8):72918
9. *Felippe P.A.N., Da Silva L.H.A., Santos M.M.A.B., Spilki F.R., Arns C.W.,* (2010), Genetic diversity of avian infectious bronchitis virus isolated from domestic chicken flocks and coronaviruses from feral pigeons in Brazil between 2003 and 2009. Avian Diseases, 54(4):191-1196
10. *Gough R.E., Cox W.J., Winkler C.E., Sharp M.W., Spackman D.,* (1996), Isolation and identification of infectious bronchitis virus from pheasants, Vet Record, 138: 208-209
11. *Herman V., Moga Mânzat R., Rămneanţu M.,* (2008), Diagnosis in infectious diseases of animals (in Romanian), (Ed.) Mirton, Timişoara, Romania
12. *Ito T., Sugino K., Satoh D., Muramatsu Y., Sano G., Sato K., Sakaguchi S., Isobe K., Sakamoto S., Takai Y., Hata Y., Shibuya K., Uekusa T., Kurosaki A., Homma S.,* (2010), Bird fancier's lung which developed in a pigeon breeder presenting organizing pneumonia.Intern Med, 49(23):2605-2608
13. *Martini M.C., Caserta L.C., Dos Santos M.M.A.B., Barnabé, Ana CS., Durães-Carvalho R., Padilla MA, Simão R.M., Rizotto L.S., Simas P.V.M., Bastos J.C.S., Cardoso T.C., Felippe P.A.N., Ferreira H.L., Arns C.W.,* (2018), Avian coronavirus isolated from a pigeon sample induced clinical disease, tracheal ciliostasis, and a high humoral response in day-old chicks. Avian Pathol, 47(3):286-293
14. *Mitrică Mărăcine D.C., Popa V., Herman V., Iancu I., Cătană N.,* (2019), Research on the frequency of some strains of avian coronavirus in an outbreak of infectious bronchitis, Proceedings of the Multidisciplinary Conference on Sustainable Development Filodiritto International Proceedings Filodiritto Edit. – Proceedings First Ed., September, 689-692.
15. *Ohtani Y., Saiki S., Kitaichi M., Usui Y., Inase N., Costabel U., Yoshizawa Y.,* (2005), Chronic bird fancier's lung: histopathological and clinical correlation. An application of the 2002 ATS/ERS consensus classification of the idiopathic interstitial pneumonias. Thorax, 60:665-667
16. *Olariu-Jurca A., Olariu-Jurca I., Coman M., Stancu A.,* (2015), Compendium de anatomie patologică practică. (Ed.) Eurobit, Timișoara, Romania
17. *Pop M., Vasiu C., Olariu-Jurca I., Olariu-Jurca A.,* (2011), Diagnostic epidemiologic şi morfoclinic în boli infecţioase la animale, (Ed.) Eurobit, Timișoara, Romania.