

MORPHOPATHOLOGICAL RESEARCH IN INFECTIOUS BURSAL DISEASE CERCETĂRI MORFOPATOLOGICE ÎN BURSITA INFECȚIOASĂ AVIARĂ

Liliana Elena TESLICI¹⁾, Iasmina LUCA¹⁾,
Oana MATEIU-PETREC¹⁾, V. HERMAN¹⁾,
A. OLARIU-JURCA^{1),*)}

ABSTRACT | REZUMAT

The study was conducted between February 2017 and April 2020, at the Discipline of Forensic Medicine and Necropsy Diagnosis of F.V.M. Timisoara. There were necropsied 238 hens, of different breeds and aged between 3 days and 6 weeks from the intensive breeding system. After examining the corpses in order to elucidate the cause of death, 32 cases showed changes in the bursa of Fabricius, leading to the presumptive diagnosis of infectious bursal disease: the bursa had a globular appearance, greatly increased in volume, pasty consistency (frequent and important as diagnostic significance - edematous lymphobursitis); the muscles on examination on the section showed small hemorrhages, the kidneys in some cases were pale, gray-yellow, with evidence of a lobular pattern and friable consistency - uric nephrosis. After evisceration, a thorough macroscopic examination of the organs under study was performed. Tissue samples (fragments) of 2 / 1.5 cm were taken from the bursa of Fabricius, thymus, spleen, cecal tonsils and kidneys which were introduced into formalin vol. 10% for fixation. The samples were fixed in 10% formaldehyde solution, where they were kept for 24 hours, after which they were processed by the paraffin method, stained by the Masson trichrome method (HEA). The histopathological preparations obtained were examined under an Olympus Cx41 microscope, with increasing objectives (6x, 10x, 20x, and 40x). In all studied cases, the disease was expressed by edematous lymphobursitis, no cases of hemorrhagic-necrotic inflammation of the bursa were identified, suggesting that the disease was caused by less aggressive variants of the Infectious bursal disease virus.

Keywords: chicken, bursa of Fabricius, infectious bursal disease, *Birnaviridae*, RNA virus

Studiul a fost efectuat în perioada februarie 2017 - aprilie 2020, la Disciplina de Medicină Legală și Diagnostic necropsic a F.M.V. Timișoara. Au fost necropsiate 238 de găini, de rase diferite și cu vârsta cuprinsă între 3 zile - 6 săptămâni provenite din sistemul intensiv de creștere. După examinarea cadavrelor în vederea elucidării cauzei morții, 32 de cazuri au prezentat modificări ale bursei Fabricius, ce conduceau spre diagnosticul prezumtiv de bursită infecțioasă: bursă cu aspect globulos, mărită în volum, consistență păstoasă, cu evidențierea pe secțiune a foliolelor bursale (leziunea cea mai frecventă și importantă ca semnificație diagnostică - limfobursită edematoasă); pe secțiune musculatura prezenta mici hemoragii, la unele cazuri rinichii erau palizi, cenușii-gălbui, cu evidențierea desenului lobular și consistență friabilă - nefroză urică. După eviscerare s-a efectuat examinarea macroscopică amănunțită a organelor luate în studiu. S-au prelevat probe de țesut de 2/1,5 cm din bursa Fabricius, timus, splină, amigdale cecale și rinichi care au fost fixate în formol 10%. Probele au fost fixate în soluție de formaldehidă 10%, unde au fost menținute 24 de ore, după care au fost prelucrate prin metoda la parafină, colorate prin metoda tricromică Masson (HEA). Preparatul histopatologic obținut au fost examinate la un microscop Olympus Cx41, cu obiective crescânde (x6, x10, x20, x40). În toate cazurile studiate, boala s-a exprimat morfopatologic prin limfobursită edematoasă, nu au fost identificate cazuri de inflamație hemoragico-necrotică a bursei, sugerând că boala a fost cauzată de variante mai puțin agresive ale virusului bursitei infecțioase.

Cuvinte cheie: pui, bursa Fabricius, infectious bursal disease, *Birnaviridae*, virus ARN

Infectious bursal disease (IBD) is an endemic, acute disease-specific to chickens, caused by a virus belonging to the family *Birnaviridae*, genus *Avibirnavirus*, whose genome consists of two segments of double strands of RNA. IBD is characterized by general and digestive disorders, especially enteric, whitish

aqueous diarrhoea, which agglutinates the pericloal feathers, and the destruction of the lymphoid organs, mainly of the bursa of Fabricius (2, 20, 23).

The disease has been identified in broilers since 1957 in the United States and was first described by Cosgrove in 1962 as "avian nephrosis".

In Romania, the disease was reported in 1967 by Adameșteanu I. and Adameșteanu Constanța (6).

IBD, along with Newcastle disease and avian infectious bronchitis, is one of the most common infectious diseases of poultry in both intensive production sys-

1) Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania”,
Faculty of Veterinary Medicine, Timisoara, Romania

*) Corresponding authors: olariujurca_adrian@yahoo.com

tems and extensive rearing systems (8, 15). These diseases cause significant losses both directly and indirectly, being favourable factors especially by immunosuppression for bacterial infections that increase losses and require additional treatments especially with antibiotics (10, 11).

Hens are receptive to hatching and up to 18 weeks the most frequently and severely affected being chicks from 6- to 8-week-old. The ages correlates well with the development of the bursa of Fabricius exchange and the period in which the level of yolk antibodies decreases (increases in relation with age) (3, 1).

The subclinical form is present in the first weeks of life and is manifested by growth delays in the chicks (9, 18). Macroscopic changes are very discreet, sometimes even inapparent. Thus, the pallor of the crest and the chin is found, some haemorrhages in the pectoral and limb muscles, bursa of Fabricius increased in the first two days of the evolution of the disease and much reduced compared to normal after 16 days. Histological examination of the bursa reveals oedema of the bursa with pronounced lysis of bursocytes in the follicles in the first 10 days after infection (13, 20) and then the invasion of the bursa with reticular and macrophage cells, followed by the installation of severe fibroplasia. The classic form of IBD is observed in chickens over 3 weeks old and is characterized by haemorrhages in various tissues and organs, including the proventriculus, granular-greasy nephrosis (IBD virus is the most important nephrogenic agent of birds) (18, 20), lipid hepatosis, splenic fibrinoid necrosis externalized by small whitish points in the mass of the organ, and the oversizing of the bursa which becomes globose, congested and haemorrhagic infiltrated. In slower evolutions, accumulations of fibrinous exudate can be observed on the surface and in the lumen of the bursa (22). The aggressive form is produced by highly virulent virus strains, which through their special antigenic structure can overcome the protection offered by standard vaccines (18, 21). These viruses multiply in the bursa follicles and produce high mortality, sometimes up to 70% (4, 14). Attention is drawn to the haemorrhages in the pectoral and limb muscles, true blood clots in the lumen of the bursa slightly enlarged in volume, and frequent proventricular haemorrhages as in Newcastle disease. The characteristic lesions in muscle, bursa and proventriculus can be observed on the same corpse or different corpses. White necropsy, or the absence of any macroscopic changes, is very common in this form of the disease (5, 9, 17, 18). Histological examination reveals necrosis of the bursal follicles, the folioles taking on a tree-like appearance and complete cell plundering of the bursal follicles (18, 20, 23). In all organs are revealed the aspects of disseminated intravascular coagulation syndrome, respectively the presence of hyaline micro-

thrombi and fibrinous emboli (5, 17, 19, 22).

MATERIALS AND METHODS

The necropsy was performed using a specific bird necropsy technique (5, 16, 17). After evisceration, a macroscopic examination of the organs was performed, establishing that 32 cases showed changes in the bursa of Fabricius, leading to a presumptive diagnosis of IBD. From these cases, samples of 2 x 1.5 cm were taken from the bursa of Fabricius, thymus, spleen, cecal tonsils, and kidneys which were immersed in formalin (10%) for 24 hours for fixation, followed by modelling and a new formalin bath for final fixation for 2 days. Subsequently, the pieces were prepared for the paraffin method. 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. Paraffin embedded tissues were sectioned at the microtome at 6µm, from each block sticking on the blade about 2-4 sections. The obtained sections were fixed on well-degreased slides with Mayer albumin. The sections glued to the slides were stained by the Masson trichrome staining modified by Ciurea with methyl blue for overall examination and the Giemsa method for cellular details (7, 16). After staining, the sections were dehydrated and mounted in anhydrous medium with Canada Balsam. The obtained histopathological preparations were examined under an Olympus Cx41 microscope, with increasing magnitudes (x6, x10, x20, x40).

RESULTS AND DISCUSSIONS

The carcasses of chickens with the presumptive diagnosis of IBD were dehydrated, with bruises and/or petechiae in the pectoral and limb muscles (Fig. 1), serous/catarrhal bursitis and uric acid nephropathy (some cases) (Fig. 2), oedematous and/or haemorrhagic bursitis (the bursa had a globular appearance, with subcapsular serous exudate), oedematous, glossy bursal follicles and in some cases with bruises and / or petechiae.

Five 2-week-old chicken corpses were dehydrated and retarded growth, wattles and combs were pale and significantly lighter than normal.

In five chickens 3-10 days-old, the bursa of Fabricius was oedematous, pink or red and serous infiltrated. When opened, the luminal surface revealed swollen, pink and glossy folds and a liquid, mucous, semi-viscous content.

Microscopically, in the bursa of Fabricius was recor-

ded lympho-cytolysis and oedema - serous exudate and subscapular, interstitial and intrafollicular bursocytolysis (Fig. 3).



Fig. 1. Chicken carcass with infectious bursal disease: general appearance



Fig. 2. Chicken carcass with infectious bursal disease: edematous lymphobursitis and obliterated ureteral uric stones

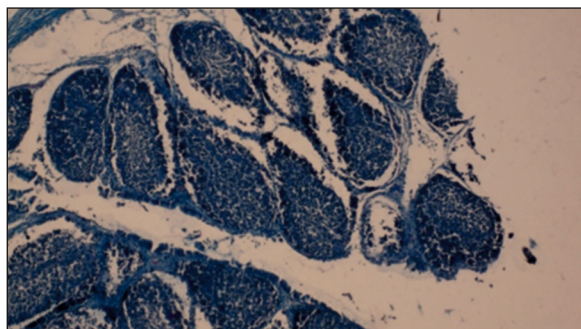


Fig. 3. Oedematous bursitis (8 days old chicken) with lympho-cytolysis: subscapular, interstitial serous exudate and intrafollicular bursocytolysis, Masson trichrome staining, 10x.

In thymus - ectasias of the interstitial capillaries - thymic congestion, the tendency to uniformize the thymic structure as a result of lymphocyte migration

from the cortical area to the medullary area (Fig. 4). In the spleen - massive lymphocyte migrations that lead to the uniformity of the white pulp structure, with the disappearance of the follicular aspect (primary lymphoid follicles-secondary lymphoid follicles) and including the red pulp (Fig. 5). In the cecal tonsils - massive lymphocyte migrations (Fig. 6).

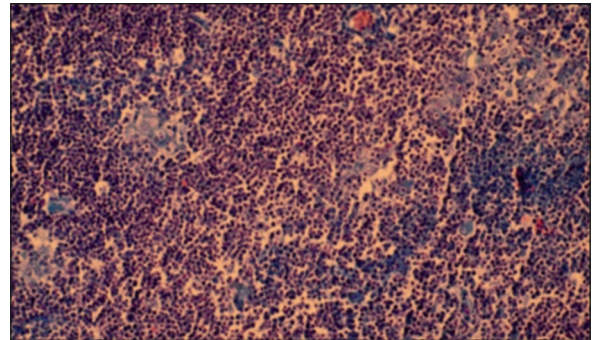


Fig. 4. Thymic congestion (8 days old chicken): ectasia of interstitial capillaries, lymphocyte migration and / or lymphocytolysis accompanied by the disappearance of the bisonal aspect C.A./M.A., Masson trichrome staining, 20x

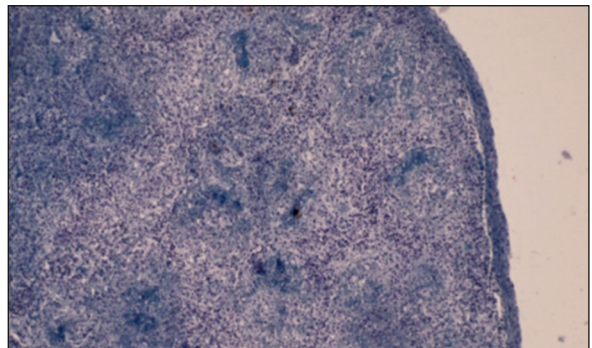


Fig. 5. Spleen (5 days old chicken): massive lymphocytomigrations, uniformity of the white pulp structure thru the disappearance of lymphoid follicles (lymph nodes), Masson trichrome staining, 20x

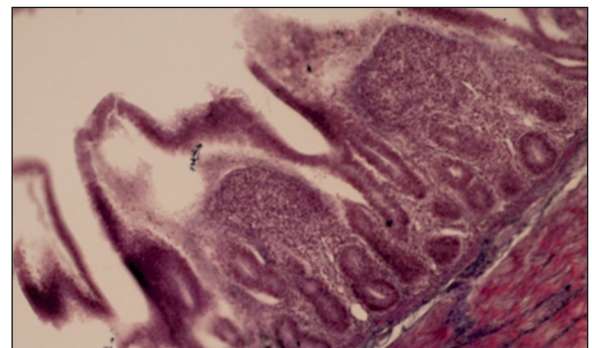


Fig. 6. Cecal amygdala (5 days old chicken): lymphocytomigration and / or massive lymphocytolysis, Masson trichrome staining, 20x



Fig. 7. Edematous haemorrhagic lymphobursitis (3 weeks old chicken): colicvation necrosis of bursal follicles, infiltration of interfollicular tissue with inflammatory cells and erythrocytes, Masson trichrome staining, 10x

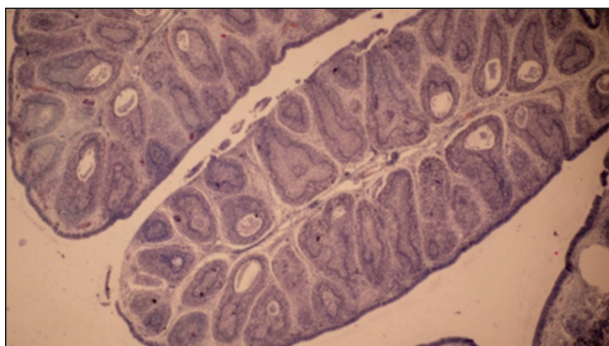


Fig. 8. Oedematous serous-catarrhal bursitis (5 weeks old chicken): de-epithelialization of bursal follicles, lymphocytolysis- cystization of bursal follicles, Masson trichrome staining, 20x

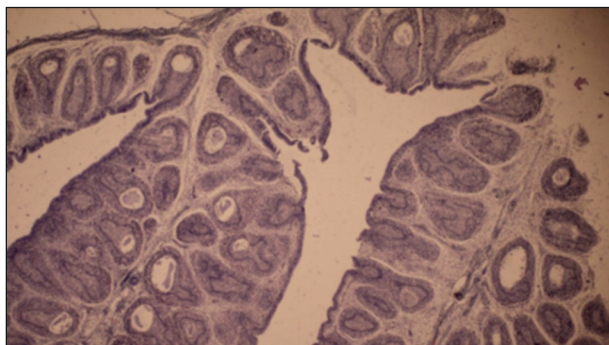


Fig. 9. Oedematous bursitis (6 weeks old chicken): cystisation of bursal follicles and interstitial fibroplasia, Masson trichrome staining, 20x

According to some authors, the pronounced lysis of bursocytes in the follicles in the first 10 days after infection and then the invasion of the reticular and macrophage cells is followed by the installation of an accentuated fibroplasia (Fig. 7) and implicitly of immunosuppression (12, 13, 23). The onset of immunosuppression favouring the appearance of Marek's di-

sease, Newcastle disease, infectious laryngotracheitis, infectious bronchitis, infectious bursal disease, inclusion body hepatitis, colibacillosis, anaerobiosis, salmonellosis, and coccidiosis (10, 18, 22).

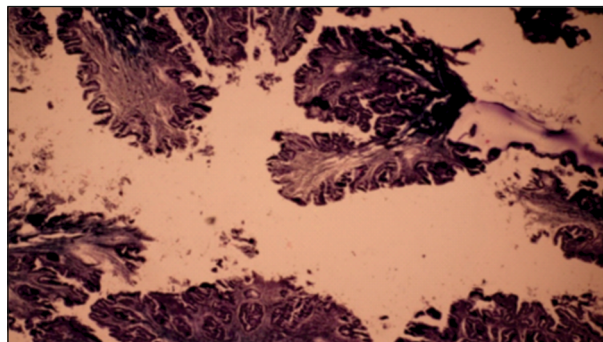


Fig. 10. Serous-catarrhal bursitis (6-week-old chicken): serous-catarrhal exudate in the lumen of the bursa, Masson trichrome staining, 10x

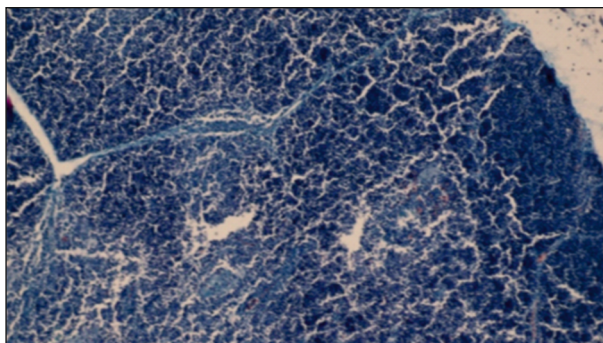


Fig. 11. Thymus (3 weeks old chicken): lymphocyte migration and / or lymphocytolysis, accompanied by disorganization of thymic architecture, Masson trichrome staining, 10x

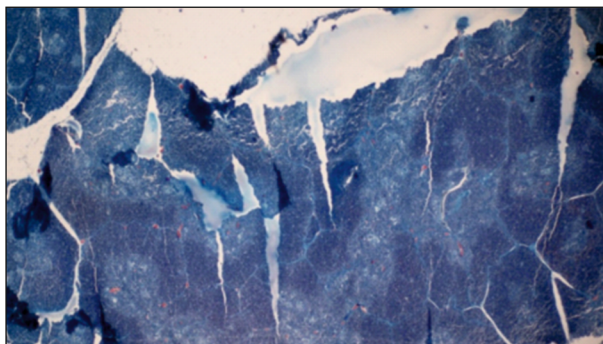


Fig. 12. Thymus (6 weeks old chicken): serous exudation and incipient intraparenchymal fibroplasia, Masson trichrome staining, 20x

At 18 chickens, aged 3-6 weeks, the bursa of Fabricius was much enlarged in volume, with a globulous appearance, and the bursal folds were swollen, oedematous, with a moist, glossy appearance and the presence in the lumen of a semifluid less viscous content -

serous-catarrrhal bursitis. Microscopically, it was observed an accentuated "melting" of the lymphocytes from most of the bursa follicles giving the appearance of cystization of the bursa of Fabricius (Fig. 8, 9, 10). In the thymus in terms of uniformization of the thymic structure produced by lymphocyte migration and / or lymphocytolysis from the cortical area to the medullary area, the boundary between the cortical area and the medullary area disappears (Fig. 11), areas of thymic exudation and fibroplasia are also observed (Fig. 12).

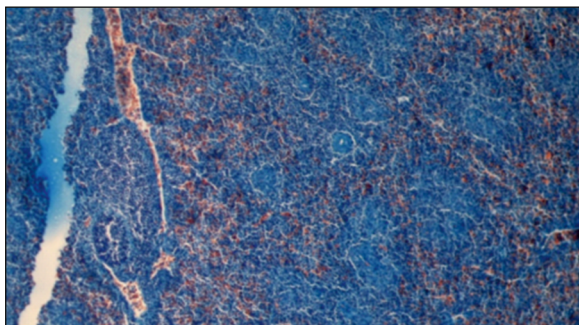


Fig. 13. Spleen (6 weeks old chicken): serous exudation, perivascular and intraparenchymal massive fibrosis, Masson trichrome staining, 10x

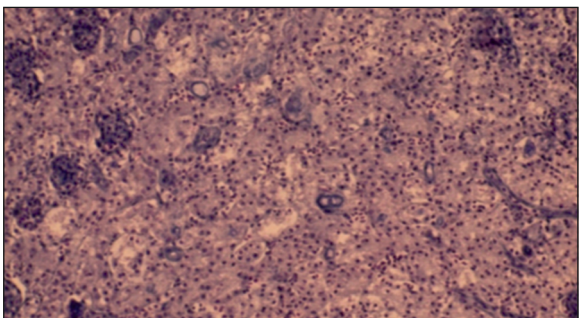


Fig. 14. Kidney (5 weeks old chicken): granular and membranous tubulonephrosis, Masson trichrome staining, 20x

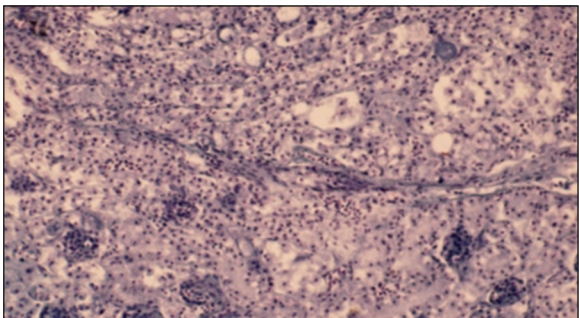


Fig. 15. Kidney (6 weeks old chicken): areas of necrosis diffused in the renal parenchyma (starry sky appearance), Masson trichrome staining, 20x

In the spleen are observed areas of serous exudation, perivascular and intraparenchymal fibrosis (Fig. 13). In the kidneys, in some areas, there are reno-

cytes detached from the basal membrane falling into the lumen, and in other areas there are numerous basophilic granulations in the cytoplasm of the renocytes - granular and membranous tubulonephrosis (Fig. 14), in some cases, there are many areas of necrosis arranged diffusely in the renal parenchyma giving the appearance of a "starry sky" (Fig. 15).

The histopathological aspects identified in poultry with IBD were described by other authors as well, however, they limited their findings to the changes of the bursa of Fabricius, without extrapolating the research to other tissues and organs (6, 12, 13).

CONCLUSIONS

In all cases studied, the disease was expressed by oedematous bursitis, no cases of hemorrhagic-necrotic inflammation of the bursa of Fabricius were identified, suggesting that the disease was caused by less aggressive variants of the Infectious bursal disease virus. Lymphoid organs from cases of infectious bursal disease undergo an extensive process of lymphocyte spoilage by lymphocyte migration and / or lymphocytolysis, areas that in most cases are replaced by fibrosis. The massive decrease of lymphocytes in the parenchyma of lymphoid organs represents the morphological substrate of immunosuppression and justifies the weakness or absence of immunological reactivity in cases of infectious bursal disease, creating the possibility of associated viral, bacterial, or parasitic diseases. Renal lesions expressed by membranous tubulonephrosis represent the organic substrate of urate salt deposition in the renal parenchyma and the installation of renal gout. Uric nephrosis and / or nephritis certify that the Infectious bursal disease virus is the most nephropathogenic biotic agent.

REFERENCES

1. Ashraf S., Abdel-Alim G., Saif Y.M., (2006), Detection of antibodies against serotypes 1 and 2 infectious bursal disease virus by commercial ELISA kits, *Avian diseases*, 50(1):104-109
2. Bercea I., Mardari A, Moga M.R., Pop M., Popoviciu A., (1981), *Infectious animal diseases (in Romanian)*, (Ed.) Didactica și Pedagogica, Bucharest, Romania
3. Cătană N., Lazău Al., Herman V., Marcu A., Rămneanu M., Ciorba D., (1996), Epidemiological, clinical and morphopathological research in infectious bursal disease. *Lucr St Med Vet Timisoara*, 29:169-171
4. Cătană N., Herman V., Popa V, Iancu I., (2014), Detection of very virulent strains of the infectious bursal disease virus. *Lucr St Med Vet Timisoara*, 47(3):10-13

5. Cătoi C., (2003), Veterinary necropsy diagnosis (in Romanian), (Ed.) Academic Press, Cluj-Napoca, Romania
 6. Coman T., (2005), Infections caused by viruses of the Birnivoridae family (in Romanian), In: coord Moga M.R., Infectious and prion diseases of animals, (Ed.) Brumar, Timisoara, Romania
 7. Cotea C., (2003), Special Histology, (Ed.) Tehnopress, Iasi, Romania
 8. Herman V., Costinar L., Pascu C., (2003), Studies in immunoprophylaxy in Newcastle disease (ND). Buletinul Universității de Științe Agricole și Medicină Veterinară Cluj-Napoca, Medicină Veterinară, 60:229-232
 9. Herman V., Moga M.R., Râmneanțu M., (2008), Diagnosis in infectious diseases common to several animal species (in Romanian), (Ed.) Mirton, Timisoara, Romania
 10. Iancu I., Popa V., Groza I., Herman V., Cătană N., (2013), The association between APEC strains and some viruses in broilers. Current Opinion in Biotechnology, 24(Suppl1):S107
 11. Imre K., Herman V., Morar A., (2020), Scientific achievements in the study of the occurrence and antimicrobial susceptibility profile of major food-borne pathogenic bacteria in foods and food processing environments in Romania: review of the last decade. BioMed Res Internat, Article ID 5134764
 12. Jubb K.V.F., Kennedy P.C., Palmer N., (1993), Pathology of domestic animals, vol I, II, III, (Ed.) Academic Press, New York, USA
 13. McGavin M.D., Carlton W.W., Zachary J.F., (2001), Special veterinary pathology, (Ed.) Mosby, USA.
 14. Moga-Mânzat R., Șofei D., Cătană N., Sarcă M., Herman V., Zecheru L., (1999), Field testing of Romanian vaccines against infectious bursal disease (in Romanian). Revista Romana de Medicina Veterinara, 9(2):187-193
 15. Olariu-Jurca A., Coman M., Olariu-Jurca I., Stancu A., Lazău A., Avram E., (2010) Morphopathological changes in avian infectious bronchitis, in the respiratory form, in chickens and aspects of histopathological diagnosis of disease, Lucrări Științifice-Medicină Veterinară, Universitatea de Științe Agricole și Medicină Veterinară" Ion Ionescu de la Brad" Iași, 53(12):453-456
 16. Olariu-Jurca A., Coman M., Olariu-Jurca I., Stancu A., (2015), Compendiu de anatomie patologică veterinară practică/ Compendium of practical veterinary pathological anatomy, (Ed.) Eurobit, Timisoara, Romania
 17. Olariu-Jurca I., Olariu-Jurca A., (2018), Necropsy diagnosis by species and syndromes (in Romanian), (Ed.) Eurobit, Timisoara, Romania
 18. Paul I., (2012), Etiomorphopathology of viruses and prion diseases in animals (in Romanian), (Ed.) Performantica, Iasi, Romania
 19. Perianu T., (2012), Treated of infectious animal diseases. Viruses and prion diseases, I (in Romanian), (Ed.) Universitas XXI, Iasi, Romania
 20. Pop M., Vasiiu C., Olariu-Jurca I., Olariu-Jurca A., (2011), Epidemiological and morphoclinical diagnosis in infectious diseases of animals (in Romanian), (Ed.) Eurobit, Timisoara, Romania
 21. Jibru I., Cătană N., Moț T., Herman V., (2002), Ergosan product use in the avian infectious bursal disease immunoprophylaxy. Lucr St Med Vet Timisoara, 35:626-629
 22. Vasiiu C., Tudor V., Vasiiu A., Predoi F., (2015), Bacterial and viral diseases in birds (in Romanian), (Ed.) Napoca Star, Cluj-Napoca, Romania.
- Withers D.R., Young J.R., Davison T.F., (2005), Infectious bursal disease virus-induced immunosuppression in the chick is associated with the presence of undifferentiated follicles in the recovering bursa, Viral immunology, 18(1):127-137.