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*Original article*

# Histopathology in diagnosis of broiler chicken and layer diseases – review of cases 1999-2010

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## Abstract

The aim of this study was to estimate the prevalence of histopathological lesions in the different organs in relation to the commercial-type and the age of birds (i.e. broiler chickens and layers). During the period 1999-2010 a total of 189 cases was submitted to the Division of Animal Pathomorphology, Department of Pathology and Veterinary Diagnostics at WULS. Most cases were found in broiler chickens (66.7%). The majority of the histopathological lesions were detected in the liver and lymphoid organs. In of 29% cases of hepatic injury pathognomonic lesions associated with inclusion body hepatitis (IBH) were found. The mean age of birds was 23 days. Among IBH cases proventriculitis (58%) was more often found than gizzard lesions (25.8%). Interestingly, we noted some intranuclear inclusions in the epithelial cells within the proventriculus. A low percentage of histopathological evidence of infectious bursal disease (IBD) was reported in chickens. The gastrointestinal tract was the second most frequent predilection site for histopathological lesions. Histopathological findings within the heart and lungs were less common and were more often seen in the upper respiratory tract. Cases of infectious laryngotracheitis (ILT) were registered in broiler chickens (3.2%, mean age 37 days) and in layers (4.8%; mean age 196 days). Lesions associated with Marek's disease, avian leukosis and fowl pox were recognized only in layers, respectively in 3.2% (mean age 176 days), 1.6% (mean age 205 days) and 1.1% (mean age 196 days) of all cases. Avian encephalomyelitis (AE) was noted only in 0.5% of all cases.

**Key words:** histopathology, poultry diseases, broiler chickens, layers, Poland

## Introduction

In veterinary medicine there is a variety of methods, which allow a correct diagnosis of poultry diseases to be established, which is not always straightforward because of their complex and diverse etiology. For many years, at the Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences

– WULS (SGGW) great importance has been attached to the usefulness of histopathology (HP) in the diagnosis of avian diseases. Despite the priority of modern techniques of molecular biology, histopathology is a reliable and economically reasonable method in terms of national conditions and can often lead to a conclusive diagnosis. It is clear that the whole picture of the disease depends on many different factors, including pathogen virulence, complicating factors

Table 1. Occurrence and localization of histopathological lesions (or diseases) in various age groups of broiler chickens.

Localization of lesions or disease diagnosed	Age groups of broiler chickens		
	group I	group II	group III
	0-10 days <sup>^</sup> n (%)	11-32 days n (%)	over 32 days n (%)
Liver and lymphoid organs (n=70)	8 (11)	45 (65)	17 (24)
IBH (n=29)	4 (14)	20 (69)	5 (17)
Bursa of Fabricious (n=20)	1 (5)	10 (50)	9 (45)
IBD (n=10)	–	4 (40)	6 (60)
Gastrointestinal tract (n=15)	–	6 (40)	9 (60)
Larynx and trachea (n=13)	–	3 (23)	10 (77)
ILT (n=4)	–	1 (25)	3 (75)
Heart (n=6)	2 (23)	4 (67)	–
Lung (n=1)	–	–	1 (100)
AE (n=1)	1 (100)	–	–

<sup>^</sup>n = number of cases. Each case was counted once and qualified for the group depending on the degree of major or pathognomonic lesions for an individual disease in the given organs.

Table 2. Occurrence and localization of histopathological lesions (or diseases) in various age groups of layers.

Localization of lesions or disease diagnosed	Age groups of layers			
	group I	group II	group III	group IV
	0-10 days <sup>^</sup> n (%)	11-84 days n (%)	85-126 days n (%)	over 126 days n (%)
Liver and lymphoid organs (n=37)	–	5 (14)	3 (8)	29 (78)
IBH (n=2)	–	2 (100)	–	–
Bursa of Fabricious (n=1)	–	1 (100)	–	–
Gastrointestinal tract (n=9)	–	1 (11)	–	8 (89)
Larynx and trachea (n=4)	–	–	–	4 (100)
ILT (n=3)	–	–	–	3 (100)
Heart (n=1)	–	1 (100)	–	–
MD (n=6)	–	–	1 (17)	5 (93)
AL (n=3)	–	–	–	3 (100)
FP (n=2)	–	–	–	2 (100)

<sup>^</sup>n = number of cases. Each case was counted once and qualified for the group depending on the degree of major or pathognomonic lesions for an individual disease in the given organs.

and the epizootic situation in specific countries. Thus, in such cases the HP may expand the knowledge of avian pathology. As far as we know, there is no documented data regarding the histopathological spectrum of avian diseases among broiler chickens and layers in Poland. Although many studies covering the appropriate diagnosis of the different diseases are available, there is a lack of available data in Polish veterinary literature concerning the role of histopathology in the detection of the pathognomonic lesions of particular avian diseases. Therefore, we decided to (1) assess the occurrence of histopathological

lesions in the different organs depending on the two farming types with respect to laying hens and broiler production and the age of the birds, and to (2) determine the frequency of the pathognomonic histopathological pattern for a particular avian disease in respective bird groups.

## Materials and Methods

The tissue samples were collected during 1999-2010 for histopathological examination at the

Division of Animal Pathomorphology, Department of Pathology and Veterinary Diagnostics, WULS. The specimens were obtained during necropsy and provided by the Division of Avian Diseases and private-practice veterinarians. The samples were fixed in 10% buffered formalin and embedded in paraffin. 4µm-thick sections were then stained using the haematoxylin and eosin method (H-E) and others if needed (e.g. Ziehl-Nielsen, PAS, Congo red stain). For further analysis we selected those cases which were attached to a completed referral form, and most tissue samples, as well as a similar set of tissues, were submitted for examination. Each case was counted once and qualified for the group depending on the major or pathognomonic lesions for a disease in the given organs. Additionally, general information was noted, i.e. age and type of bird in commercial production. Based on the production cycle in the poultry industry the following age groups were established:

- broiler chickens: group I: 0-10 days, group II: 11-32 days, III group: more than 32 days;
- layers: group I: 0-10 days, group II: 11-84 days, group III: 85-126 days, group IV: more than 126 days.

## Results

In 1999-2010 419 histopathological (HP) examinations of samples taken from different bird species were performed. Of all cases, 55% were found in poultry, 25% in pigeons, 14% in psittacine and 4% in passerine birds, and 2% in wild birds. Among poultry, most cases were derived from broiler chickens (66.7%; n=126) and layers (33.3%; n=63). The occurrence of pathological changes depending on the localization and the age group of the broiler chickens and layers are shown in Table 1 and Table 2. The majority of lesions were localized within the liver. All cases of hepatic injury were associated with lymphoid tissue damage (spleen and/or bursa of Fabricius and/or thymus) in broiler chickens as well as in layers (55.6% and 58.7% respectively). Histopathological examination of the liver is presented in Figs. 1A-C. In many samples of liver (29%) obtained from all birds with hepatic injury large basophilic intranuclear inclusion bodies in the hepatocytes, compatible with inclusion body hepatitis (IBH), were revealed, whereas bacterial emboli, granulomas and fungal infection (case in 5-day-old broiler) were rare. The mean age of birds with IBH was 23 days. Among 58% cases of IBH proventriculitis was noted and in 11% cases of these cases basophilic intranuclear inclusion bodies in glandular epithelial cells were recognized (Fig. 1G). Gizzard lesions were noted in 25.8% cases of IBH.

The histopathological changes in lymphoid organs are illustrated in Figs. 1D-E. Additionally, deposits of amyloid were seen in the spleen (in 2 layers over 126 days old).

Among the bursa of Fabricius samples collected from broiler chickens (n=20) in the direction of infectious bursal disease (IBD) we noted marked atrophy of the lymphoid follicles. Additionally, in 10 samples (7.9% of all broilers) characteristic histopathological lesions for IBD (Fig. 1F) were noted. In 60% of cases of IBD we noted moderate to marked proventriculitis.

Histopathological lesions observed within the gastrointestinal tract were most common in birds of age groups III (Table 1) and IV (Table 2). A high number of coccidian oocysts invading cells lining the small intestine was noted in only two broiler chickens (Fig. 1H).

Most of the findings within the larynx and trachea were inflammatory in nature. Syncytial cells and Seifried inclusion bodies, considered pathognomonic for infectious laryngotracheitis (ILT), were also present (Fig. 1I). ILT cases were noted in broiler chickens (3.2%, mean age 37 days) and in layers (4.8%, mean age 196 days).

Among broiler chickens significant lesions in the heart (Fig. 1J) were relatively frequent. In the lungs mild to moderate exudative and proliferative bronchopneumonia (Fig. 1K) was observed. Significant lesions in the lungs were noted only in a broiler chicken over 32 days old and included granulomatous pneumonia.

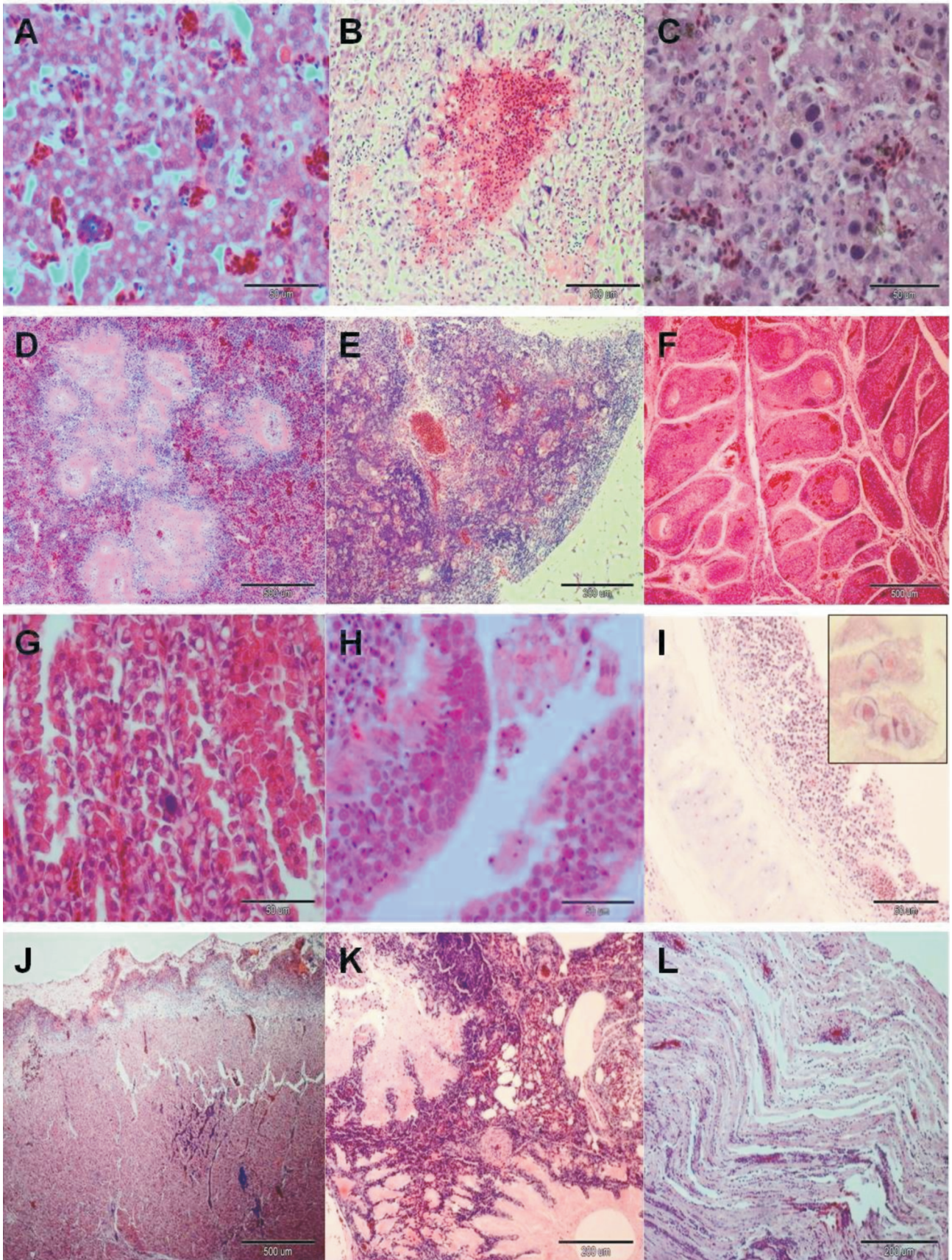
Neoplastic diseases of poultry: Marek's disease (MD) and avian leukosis (AL) were diagnosed in 3.2% and 1.6% of all surveyed birds, respectively. These cases were noted only in layers (at mean age 176 days and 205 days, respectively). Pathognomonic findings for MD in the sciatic nerve are shown in Fig. 1L.

Histopathologically, in 1.1% of all cases marked inflammatory skin lesions and eosinophilic intracytoplasmic inclusion bodies (Bollinger) were observed, which were characteristic of fowl pox (FP). These cases concerned only layers at mean age 196 days.

Lesions in the central nervous system (CNS), indicating avian encephalomyelitis (AE) were recognized in 0.5% of all cases.

## Discussion

The crucial role that histopathology plays in diagnostic avian pathology is undisputed. Although HP is an outdated method, it is still performed in the first instance and helps to choose more specific diagnostic techniques to obtain a final diagnosis. By indicating



the presence of pathognomonic inclusion bodies, infectious agents (parasites, fungi) or neoplastic lesions, HP significantly contributes to an immediate diagnosis and narrows the further differential diagnosis. The present study was conducted to provide information regarding the prevalence of histopathological lesions within organs obtained from commercial broiler chickens and layers, which had died from various causes, and to compare lesions depending on the bird age. There is a lack of national data on similar analysis, which makes it impossible to compare our results. It is not surprising that the majority of analyzed material was derived from older birds rather than from chicks, which may be partly explained by the fact that the chicks are protected by maternal antibodies transmitted from immunized layers. On the other hand, the number of cases may be underreported, because material from birds already showing clinical signs is usually supplied for histopathological examination. Besides, a normal range of chick losses can be expected, particularly during the first week after hatching (Borzemska 2011).

The present results and those reported by other authors indicate that most of the diseases in broiler chickens and layers were associated with liver failure (Singh et al. 1996, Supartika et al. 2006). In advanced cases this organ was enlarged, and had changed colour and therefore probably hepatic samples were most often sent to the histopathology laboratory for this reason. In accordance with the data in the literature hepatic fungal infection was rarely found. We demonstrated this in one case of a 5-day-old chick, similarly to a study which described a higher sensitivity to invasive fungal infection among embryos, chicks and immunocompromised birds (Gwatkin 1986).

Granulomatous inflammation in the liver is relatively frequent and has a multifactorial basis, most often are caused by *Mycobacterium* sp., *Enterobacteriaceae* sp. or *Eubacterium tortuosum* and fungi (Supartika et al. 2006). However, in the present study they were quite rare. On the basis of additional histochemical methods it was confirmed that they were not caused by mycobacteria and fungi.

As is shown in the present study and in published data, one of the most important factors in liver pathology is fowl adenovirus infection, especially that caused by group I adenoviruses, which is attributed to IBH. For example, serological studies conducted in Poland in 1986 recorded adenovirus infection in 40% of examined birds, and in 1990-1991 it was found in 85.7%. Adenoviruses were isolated from 57.1% of birds (Samorek-Salamonowicz et al. 1990). The most frequently isolated serotypes could be associated with IBH. We noted IBH cases in 16.4% (n=31) of the total; however, in Polish literature there are no studies based on the effectiveness of HP in the diagnosis of IBH. The age of birds was similar to data in the literature (Singh et al. 1996). Interestingly, we noted some cases with pathognomonic intranuclear inclusions in the glandular cells in the proventriculus, and they were generally often detected also in the gizzard (Ono et al. 2003).

Similarly to data in the literature deposits of amyloid were extremely rare among *Galliformes*; however, they have been described in layer hens (Nakamura et al. 2006).

Additionally, all our cases of liver failure presented an involvement of the lymphoid systems, therefore lymphoid organs were also the most frequently affected organs in broiler chickens and layers. This is probably due to the fact that the lack of lymph nodes in most poultry species increases the importance of liver and lymphoid organs in resistance to various diseases. In the present study we paid attention to the role of HP in assessment of bursa of Fabricius specimens for confirmation of IBD, also known as Gumboro disease. Among broiler chickens with significant bursal damage, half of them were associated with IBD, i.e. only 7.9%. Similar results were obtained by Khan et al. (2009) on the basis of history and detailed postmortem pathological lesions, but some authors emphasized that IBD is more common (Adamu et al. 2009). The birds were in the range from 11-32 days and over 32 days old; similar findings were obtained by others (Khan et al. 2009). Proventriculitis diagnosed in some IBD cases was in accordance with data



Fig. 1. Histopathological lesions within avian organs. H-E method. A) Liver: fatty degeneration of hepatocytes, bacterial emboli; 400x; B) Liver: areas of necrosis surrounded by multinucleated giant cells, 200x; C) Liver: a coagulative necrosis and large basophilic intranuclear inclusion bodies in hepatocytes compatible with IBH, 400x; D) Spleen: coagulative necrosis, lymphocyte depletion, 40x; E) Thymus: decrease in lymphocyte density, medullary necrosis, hyperaemia, 100x; F) Bursa of Fabricius: lymphocyte depletion, necrosis and hemorrhages in follicles, interstitial oedema – lesions characterized for IBD, 40x; G) Proventriculus: large basophilic intranuclear inclusion bodies in hepatocytes compatible with IBH, 400x; H) Duodenum: coccidiosis; numerous coccidian parasites in various stages within epithelial cells of atrophic villi, mononuclear cell infiltrate, intraluminal cellular debris, 400x; I) Heart: fibrinous pericarditis; fibrinocellular exudate and bacterial colonies on surface, 40x; J) Lung: interstitial inflammation, peribronchial and perivascular lymphocytic infiltrates, fibrinous exudate in bronchial lumen, 100x; K) Trachea: inflammatory infiltrates and eosinophilic inclusion bodies in epithelial cells (in small frame) considered pathognomonic for ILT, 400x and 1000x; L) Sciatic nerve: lymphoid cell infiltration characteristic of MD, 100x.

in the literature (Pantin-Jackwood et al. 2004). It should be noted that the histopathological approach to IBD has the advantage of allowing for diagnosis of the subclinical form of the disease, when no clinical signs of infection are observed, but the IBD virus leads to immunosuppressive effects (van den Berg et al. 2000).

In this study, the second important localization of lesions was the gastrointestinal tract. The majority of these cases were recognized in older birds. This is in accordance with data in the literature which describes many contributory factors for gastrointestinal disorders among older birds; however, some of the results of natural and experimental bacterial infections of the avian digestive tract suggest that younger birds are more susceptible (Gwatkin et al. 1986).

Coccidiosis is the most common parasitic disease of the gastrointestinal tract of poultry (Adamu et al. 2009). Investigations conducted in the district of Olsztyn (Poland) lasting 6 years indicated coccidiosis in 0.04% birds, ranked 3<sup>rd</sup> among poultry diseases (Radkowski et al. 1996). In the present study such cases occurred occasionally. It seems that suspected cases of poultry coccidiosis were first examined using parasitological tests and further investigations were not conducted. Interestingly, in the present survey oocysts were reported more frequently within the small intestine than the colon, whereas the histopathological study conducted by Soomro et al. (2001) demonstrated greater invasion of caecal versus small intestines.

Generally, lesions in the upper respiratory tract including inflammatory process were diagnosed as non-specific tracheitis and, according to the literature, were commonly found in poultry in Poland and world-wide (Radkowski et al. 1996, Chacón et al. 2007). Additionally, in the present study a few cases of ILT were noted, occasionally in layers, but some authors emphasize that these cases are more frequent (Chacón et al. 2007); however, in the cited paper a definitive diagnosis was based on PCR or ELISA. There are some reports from the literature which indicate a highly comparable specificity between the histopathology and PCR or immunofluorescence test in the diagnosis of ILT (Goodwin et al. 1991, Humberd et al. 2002). For many years traditional virus isolation has been considered a gold standard for ILT diagnosis; however, it is a long-term and expensive method for routine diagnostics. Recent note that expedited histopathology and molecular detection have become the new gold standards for ILT diagnosis, since they both allow for a more rapid and effective approach (Humberd et al. 2002, Chacón et al. 2007). The mean age of affected birds was similar to data in the literature (Koncicki and Minta 2011a).

According to older data most histopathological changes of the heart and lung were inflammatory in nature and occurred predominantly in young birds, ex-

cept in the case of granulomatous inflammation of the lung (Timurkaan et al. 2008).

In our study we indicated cases of classic Marek's disease, according to the generally accepted histologic criteria in the literature (Payne and Biggs 1967). MD occurred in 3.2% of all surveyed birds, and only in layers. These results are similar to findings obtained in other studies, where peripheral nerve infiltration was observed only in layers (2.8%) (Wieliczko et al. 2002). In another retrospective study MD was noted less frequently (0.1%) in chickens from the north-east of Poland (Radkowski et al. 1996). Interestingly, our results were similar to those (3.03%) obtained in a histopathological survey conducted on other continent (Balachandran et al. 2009). These authors emphasized that MD was much more common in layers; however, in their work the proportion of layers in the flocks studied was greater. The mean age of layers with MD in the present review was similar to data in the literature (Wieliczko et al. 2002, Balachandran et al. 2009).

Similarly to other research the incidence of avian leukosis (AL) among all birds (up to 2%) was low and also less frequently noted than MD (Balachandran et al. 2009); however, in some reports AL was even more rare (Radkowski et al. 1996). In our own study, the mean age of layers was in agreement with an earlier report (Balachandran et al. 2009).

In the present study the histopathological lesions associated with cutaneous fowl pox (FP) were consistent with the literature data (Nakamura et al. 2006, Moayyedian et al. 2008). The incidence of FP was particularly rare and noted only among layers over 126 days old. The mean age of birds was similar to data in the literature (Moayyedian et al. 2008). However, some authors reported a higher incidence of FP, for example 4.64% and 19% (Fallavena et al. 2000, Adamu et al. 2009). Thus, based on our own observations and those of other authors, we suppose that macroscopic changes of skin from the slaughtered birds are not specific, and therefore were not sent for histopathological examination for a precise diagnosis (Fallavena et al. 2000, Moayyedian et al. 2008). Moreover, the climatic conditions of the country and exposure to insects play a significant role in the spread of FP. Avian pox geographic distributions have tended to be limited to localized regions even within continents (van Riper and Forrester 2007, Adamu et al. 2009).

Until 2003, the occurrence of avian encephalomyelitis (AE) in broiler chickens in Poland was extremely rare and always as a consequence of the discontinuing of prevention in the parent flock. For example, in the dispensary of the Division of Poultry Diseases at Faculty of Veterinary Medicine, WULS, in 1978-2006 only a few cases of AE in broiler chickens were noted, similarly to the present study (Dymacz et

al. 2007). Histopathological lesions in the central nervous system (CNS) were not always manifested by clinical symptoms and macroscopic changes in the brain that are visible during autopsy. The age of birds in the present study did not differ from data found in others (Jana et al. 2005, Dymacz et al. 2007).

Although the present study does not include an epidemiological aspect, we conclude that the occurrence of histopathological lesions and the mean age of broiler chickens and layers mainly did not differ from literature data. The liver and lymphoid organ involvement was most commonly noted among both broiler chickens and layers. Older birds were most often affected. IBH was the most common disease recognized by HP. In the present study we paid attention to the important cognitive value of histopathological examination in many diagnostic situations. Although pathomorphological findings and their typical location are well known from the literature and textbooks, we demonstrated that some lesions may be found in other, less usual organs, e.g. the surprising presence of inclusion bodies in the proventriculus among IBH cases. Through the application of histopathology in diagnostic process in poultry diseases it is often possible to obtain correct and rapid diagnosis using a well-planned procedure and provision of appropriate tissue samples.

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