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D. Berezhna, PhD Stud,
Center for Modern Diagnostics "Bio-Test-Laboratory", Kyiv
I. Ivashchenko, PhD
Taras Shevchenko National University of Kyiv, Kyiv

IDENTIFICATION OF MAREK'S DISEASE IN POULTRY FARMS OF UKRAINE AND ESTABLISHMENT THE FORMS OF DISEASE

Marek's disease virus is one of a pathogen who remains to cause a significant losses in poultry veterinary medicine in all over the world. In this paper, Marek's disease was identified in 234 of 304 observed samples by histological diagnosis and established the dominant manifestation of the disease. The main form that was detected in positive samples was classic manifestation of MD. The identification of Marek's disease and different forms in poultry flocks pointed on circulation of herpes virus and the existence of virulent and highly virulent variants of the virus in poultry farms of Ukraine.

Key words: Marek's disease virus, histological diagnosis.

Introduction. Marek's disease (MD) is the most widespread lymph proliferative disease of chickens and turkeys. The disease is characterized by the development of malignant lymphomas in visceral organs (acute form) and nerve damage (classical form). Thus, the annual loss of MD in global poultry industry is estimated at 1-2 billion dollars [1]. World Society of Agriculture and Food (FAO) has about 2002 the largest poultry farms, containing 45 billion broilers and laying hens and in addition 57 million tons of eggs. Thus, the poultry industry adds global budget for 100 – $ 200 billion, Marek's disease virus in turn reduces it to 1%. However, the trend of Marek's disease virus and industrial rearing of poultry is not always observed in such way. By 1960, when the industrial poultry breeding was intensified, existing variety of Marek's disease virus showed pathogenicity in a very mild form. Thus, since the first case of diagnostic Marek's disease was noticeable change in the typical clinical manifestations [5]. The list of chronic polyneuritis (multiple peripheral nerve hypertrophy) gradually joined by visceral lymphoma (formation of tumours in organs such as the heart, liver, kidneys, lungs, etc.) more acute transient paralysis, immunosuppression, brain swelling and dermatitis. However, common symptoms of the disease with lesions of different genetic variants of the virus remains paralysis and chronic inflammation that occurs directly in response cytolytic infection of B- cell proliferation, manifested as lymphoma and degenerative changes in the form of arterial atherosclerosis [6].

Another factor that directly influenced on the evolution of Marek's disease pathogenicity was the creation and using vaccines. Thus, the first vaccine was created on the base of attenuated strains (HPRS- 16) of Marek's disease virus [1]. Soon, the prevention was perfected with the development of new vaccines based on the strain that genetically related to the herpes virus of turkeys (HVT) [2]. Mass vaccination of poultry decreased the percentage of losses up to 99% and created the major barrier of protection.

It should be noted that birds losing from Marek's virus was quickly stopped after vaccine using based on strain of HVT in the early of 70's. But success maintained only to the end of 70's and loss was recovered by the emergence of more virulent variants of the virus. But, the new bivalent vaccine consisting of HVT and strain from the second serotype of MDV (MDV -2) have become a response to the virus aggression. A new approach to vaccination of poultry allowed to keep the pressure from the virus a short period of time. After a relative lull in the mid of 90s, the losses in the poultry industry gained momentum in the answer of the emergence of new highly virulent strains of Marek's disease. Thus, the industry has been forced to take an empirical approach by introducing more effective vaccine, namely CVI988. Displayed vaccine was based on a first serotype of the virus (MDV-1) [3]. Used immunization strategy proved very successful way and allowed to control losses in the industrial poultry breeding. Nevertheless, the experience of previous years, tells us that as more stable control strategy we have as sooner we get the following changes "shift" in virulence by the emergence of new, more virulent variants of the virus. The vivid proof of this theory is the recent emergence of virus isolates that show a very high level of pathogenicity in chickens that were vaccinated with CVI988 [4].

Currently, the disease is controlled by the highly efficient mono-, bi -and polyvalent vaccines. However, Marek's disease virus remains exemplary model of the evolution of virus virulence during the past 60 years and continues to move towards its increase. Pathogenesis and disease manifestation is very different and depends on various factors, namely the serotype and patotype of virus, from genetic conditions of poultry, age of infection, the immune system, and welfare of flocks.

Currently, distinguish 3 main forms of Marek's disease. Thus, an acute form manifests as malignant tumours in visceral organs (liver, spleen, kidneys, lungs, stomach), the classical form – lymphocytes proliferation in pereferiynyh nerves and mixed, in which tumours are localized in both nerves and visceral organs. The development of mixed forms cause by Marek's disease virus pointed on the circulation of the virulent and highly virulent genetic variants of the virus. Of course, the classical method determination of the pathogenicity is a selection of agent, inoculate susceptible host by this agent and take the same disease, which is characterized as the control samples. However, takes in mind the manifestation of the disease, the time of infection and the presence or absence of a vaccine can talk about the presence of virulent strains of MD.

Thus, the aim of our study was to identify the Marek's disease on poultry farms Ukraine with current manifestations.

Materials and methods. The analyse of the pathological condition of samples from 117 poultry farms and sampling was carried out in the laboratory of pathological anatomy in the laboratory of diagnostic centre of "BioTestLaboratory". The study was beginning in 2009 up to 2012 and took place in different regions of Ukraine. For studies was selected bird with such external clinical signs of disease as: lameness, paralysis of the limbs and wings, change of iris colour and it's shape and size, blindness poultry and with the sudden death. Bird with such pathological changes as diffuse focal thickening of the nerve roots of the lumbar and brachial plexus, which become dull gray swelling in the lungs, kidneys, liver, heart, spleen, gonads, glandular stomach, muscle, liver congestion and spleen, and thickening of the walls of the glandular stomach were taken for histological examination. The size of selected samples was 2-4 cm selected materials immediately transferred to a 10% solution of formalin. Container with sampled was labelled in such follows: date of sampling, age of birds, a household name, input the number in the LTD. Sectional material for histological examination left in tanks of formaldehyde at room temperature.

Fixation of tissue samples was performed by immersion in 80 ml of 10% formalin solution and holding in the microwave for 30 seconds at a power of 400 W with a water load of 400 ml. After initial fixation was performed by immersing the dehydrated tissue samples in 80 ml of 96% ethanol and exposure in the microwave for 10 min at 100 W power [10]. Then cut tissue samples (width of cut was less than 1.5 cm), taking into account the structural features of each body and the presence of visible lesions and placed in plastic cassettes Turboflowe [11].

After fixation was performed pre-processing studied tissues using automated station MicromSTP- 120. To prepare paraffin blocks, cooling the samples and filling was used the "MicromEC 350" station [81]. Preparation of thin sections of pathological material flooded in paraffin was performed using a microtome "Microm HM 340 E," and knives for histological studies "Sec- 130". Cutting was done by setting "step" microtome 50 microns, and for the manufacture of tissue samples – 5 microns. Slices of tissue samples were transferred from the water bath (water temperature 39 °C) directly into glass slides [11].

Made histological sections were kept for 1-2 hours at temperature of + 37° C for drying. Painting histological sections was performed hematoxylin and eosin solutions [81].

The sample's microscoping was carried out by an increase in the 100 and 200 times using appropriate lenses and an increase in 1000 using immersion lens microscope Axioskop 2 plus (CarlZeiss).

Results and Discussion. In 68% of the clinical cases were reported substantial polymorphic lymphoid irregular infiltrates that located in the perivascular and parenchyma of the body (Fig.1). It should be emphasized that the degree of substitution of the liver parenchyma intractable tumours ranged from 60 to 80% of body tissue.

Compared to the frequency of changes in the incidence of intractable liver tumours caused by Marek's disease virus was significantly lower. Thus, polymorphic lymphocytic infiltrates in the lungs detected only in 41 % of the cases whereas intractable tumours in the kidneys revealed only 30 % (Fig.2).

Often in studies recorded changes in the glandular part of the stomach (80 % of all samples). Taking into account poly-etiological cause of this disease (infectious bronchitis virus effect, Gumboro disease virus, poisoning, etc..), we took into account only those cases of proventrykulus in which changes were found not only in the glands and mucosa, but also in muscular glandular mucosa of the stomach. It should be emphasized that the localization of infiltrates in the muscular membrane as the stomach (15% of cases) and intestinal (detected in 32% of cases) is characterized by the classic signs of Marek's disease virus as polymorphic lymphocytic infiltrates in the peripheral nerves.

It should be added that tumours caused by Marek's disease virus in the tissues of the nervous system were found in 32% of the sample studied.

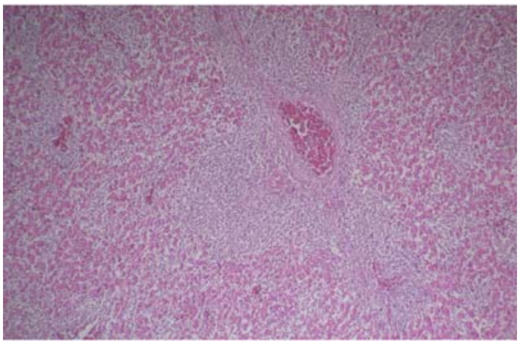


Fig. 1. Liver of damaged broiler in age of 45 days by MDV.
Heavy infiltration of proliferating mixed lymphoid cells in a liver. H&E. x100

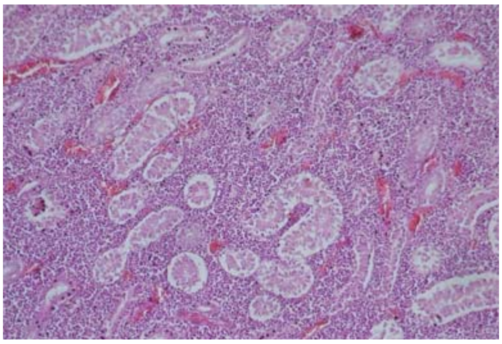
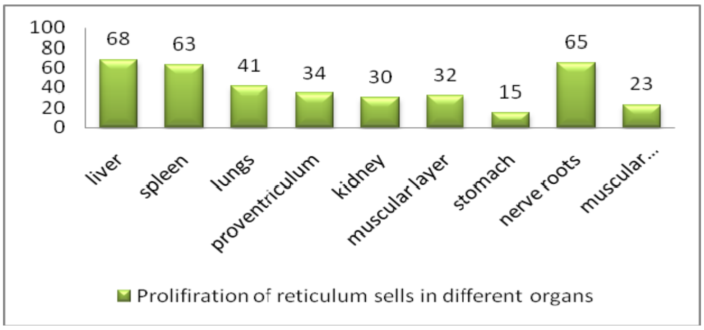


Fig 2. Heavy infiltration of proliferating mixed lymphoid cells in a kidney. H&E. x200

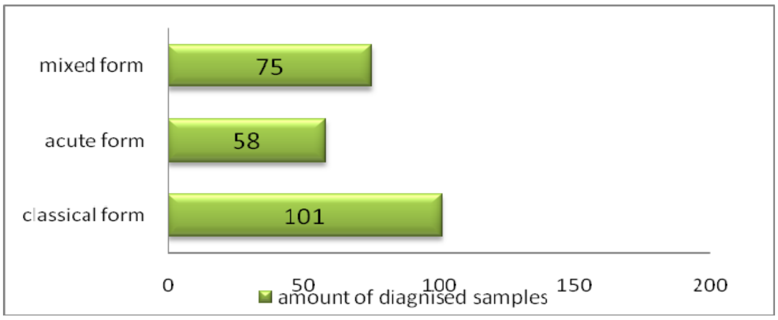
Thus, the most often tumors was met in the liver – 68%, peripheral nerves – 65%, spleen – 63%, in a somewhat lesser extent in the lung – 41% and glandular stomach – 34% (Table 1).

Table.1. **Damaged visceral organs and peripheral nerves by Marek's disease virus**



I want to emphasize that the Marek's disease was identified in 234 samples from 117 farms of Ukraine. With the help of histological tools of diagnostic, it was determined that the classic form of disease was identify in 101 sample, while mixed form in 75 studied cases. It should be noted that the mixed form of Marek's disease was detected in 58 of observed samples.(Table 2).

Table. **2 Forms of Marek's disease manifestation in observed samples**



Thus, by pathomorphological and histological tools of diagnostic was analyzed 304 samples from 117 poultry farms Ukraine from 2009 up to 2012. Marek's disease was identified in 234 samples, that pointed on the circulation of this pathogen in Ukraine. Given the age of the birds, livestock vaccination, cross and nature of lesions in various organs was established that the dominant form of expression is the classic form of the disease, in which all visceral organs of birds are affected. Number of acute and mixed form in observed cases concerning about existence of virulent and highly virulent strains of Marek's disease within our state.

References

1. Morrow C. Fehler F. Marek's disease a worldwide problem. In Marek's disease, An Evolving Problem / Davison F., Nair V. // Elsevier Academic Press. – 2004. – P.49-61.
2. Witter R.L. Increased virulence of Marek's disease virus field isolate. // Avian Dis. – 1997. – Vol. 41. – P. 149–163.
3.Nair, V. Evolution of Marek's disease–a paradigm for incessant race between the pathogen and the host. // Vet. Journey. – 2005. – Vol. 170. – P. 175–183.
4. Osterrieder N., Kamil J.P., Schumacher D., Tischer B.K., and Trapp S. Marek's disease virus: from miasma to model. // Nat. Rev. Microbiology. – 2006. – Vol. 4. – P. 753–761.
5. Atkins, K. E., A. F. Read, N. J. Savill, K. G. Renz, S. W. Walken-Brown, and M. E.Woolhouse. Modelling Marek's disease virus (MDV) infection parameter estimates formortality rate and infectiousness. // BMC Veterenary Research. – 2011. – Vol. 7.
6. Nair, V., and H. J. Kung. Marek's disease virus oncogenicity: molecular mechanisms, and V. Nair (ed.), Marek's disease, an evolving problem. // Elsevier, Oxford, United Kingdom. – 2004. – Academic Press – P. 32-48.
7. Witter, R.L. Classification of Marek's disease virus according to pathotype-philosophy and methodology.// Avian Pathology. – 2005. – Vol. 31. – P. 525-538.
8. Witter, R.L. Very virulent Marek's disease viruses: Importance and control / R.L. Witter // In Proc 18th World's Poultry Congress // World's Poultry Congress, Nagoya, Japan. – 1988. – P. 92-97.
9. Norman L J Ross, R. Wadekar, A. Lopes, A. Dagnall. Methylation of two Homo sapiens-specific X-Y homologous genes in Klinefelter's syndrome (XXY). // American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. – 2006. – Vol. 5. – P. 544 – 548.
10. Bacon L.D, Hunt H.D, Cheng HH: A review of the development of chicken lines to resolve genes determining resistance to diseases. // Poult Scientist. – 2000. – Vol. 79(8). – P. 1082-1093.
11. Fredericksen T.L, Longenecker B.M, Pazderka F, Gilmour DG, Ruth R.F. T-cell antigen system of chickens: Ly-4 and Marek's disease. // Immunogenetics. – 1977. – Vol.5. – P. 535-552.
12. Kaiser P, Underwood G, Davison F. Differential cytokine responses following Marek's disease virus infection of chickens differing in resistance to Marek's disease. // J Virol. – 2003 – Vol. 77(1). – P. 762-768.
13. Churchill, A.E. Immunization against Marek's disease using a live attenuated virus / A.E Churchill, L.N. Payne, and R.C. // Chubb // Nature – 1969. – Vol. 221 – P. 744-747.
14. Witter, R.L. Protection by attenuated and polyvalent vaccines against highly virulent strains of Marek's Disease virus / R.L. Witter // Avian Pathol. – 1982. – Vol. 11.-P. 49-62.
15. Rispens, B.H. Control of Marek's disease in the Netherlands. I. Isolation of an avirulent Marek's disease virus (strain CVI988) and its use in laboratory vaccination trials / B.H. Rispens [et al] // Avian Dis. – 1972. – Vol. 16. – P. 108-125.

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Д. Бережна, асп.
Центр сучасної діагностики "Біо-Тест-Лабораторія", Київ,
І. Іващенко, канд. біол. наук
КНУ імені Тараса Шевченка, Київ

ІДЕНТИФІКАЦІЯ ХВОРОБИ МАРЕКА НА ПТАХОФЕРМАХ УКРАЇНИ ТА ВИЗНАЧЕННЯ ФОРМИ ЗАХВОРЮВАННЯ

Вірус хвороби Марека один із патогенів, який викликає значних економічних втрат у ветеринарії птахівництва в усьому світі. В роботі, хвороба Марека була ідентифікована в 234 зразках з 304 досліджуваних. За допомогою гістологічного методу діагностики встановили, що домінантною формою прояву була класична форма. Ідентифікація хвороби Марека в різних господарствах України з різною маніфестацією, вказує на циркуляцію збудника і на різну міру патогенності самого збудника.

Ключові слова: Вірус хвороби Марека, гістологічна діагностика.

Д. Бережна, асп.,
Цент современной диагностики "Био-Тест-Лаборатория", Киев,
И. Иващенко, канд. биол. наук
КНУ имени Тараса Шевченк, Киев

ИДЕНТИФИКАЦИЯ БОЛЕЗНИ МАРЕКА НА ПТИЦЕФЕРМАХ УКРАИНЫ И ОПРЕДЕЛЕНИЕ ФОРМЫ БОЛЕЗНИ

Вирус болезни Марека – один из патогенов, который вызывает значительные экономические потери в ветеринарии птицеводства во всем мире. В работе, болезнь Марека была идентифицирована в 234 образцах из 304 исследуемых образцов. С помошью гистологического метода диагностики устновлено, что доминантной формой оказалась классическая форма заболевания. Болезнь Марека идентифицированная в разных хозяйствах Украины, что указывает на циркуляцию возбудителя и на разную сепень патогенности самого возбудителя.

Ключевые слова: вирус болезни Марека, гистологическая диагностика.

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O. Boyalska, researcher center
SI "Zhytomyr regional laboratory center of State Sanitary and Epidemiological Service of Ukraine",
Taras Shevchenko national university of Kyiv, Kyiv,
I. Kyrychuk, researcher depatment
Main Department of State Sanitary and Epidemiological Service of Ukraine in Zhytomyr region, Zhytomyr,
O. Shpyta, researcher depatment
Zhytomyr interregional management of Main Department of State Sanitary and pidemiological Service of Ukraine, Zhytomyr,
A. Boyko, Prof.
Taras Shevchenko national university of Kyiv, Kyiv

RETROSPECTIVE ANALYSIS OF INFLUENZA-INDUCED MORBIDITY IN POPULATION OF ZHYTOMYR REGION DURING 1999-2011

The epidemiological analysis of morbidity of influenza population Zhytomyr region for 13 calendar years was carried out. Dynamic of morbidity was characterized by periodic decrease and increase. The biggest data of morbidity of influenza of population from 1999-2011, were in the 1st quarter in February. The epidemic increasing of incidence in the epidemic season 2009-2010 was observed in October-December. Indicators of influenza of Zhytomyr region population coincide with the course of disease in Ukraine in general, and in some years over and above the Republican approximately 1,5 – 2. Morbidity of influenza Zhytomyr region populations has winter-spring seasonality and cyclic recurrence.

Key wods: flu, illness, seasonal, cyclical disease.

Introduction: Influenza viruses are the most prevalent pathogens of human respiratory infections and one of the most significant because they cause to high morbidity and mortality [1, 2].

The rapid pace of evolution of influenza viruses by various selection pressures, the production of novel genotypes through reassortment following mixed infections and their ability to constantly adapt to new avian and mammalian species, which makes monitoring and predictions influenza outbreaks is particularly difficult [3, 4, 5].

In the United States approximately 36,000 deaths occur annually following influenza infection. There is concern about the continuation of zoonotic infections of highly pathogenic avian influenza H5N1 [6, 7]. The worst influenza outbreak in recorded history, the so-called 'Spanish' influenza pandemic of 1918–1919 [8].

Pandemics of influenza virus appeared sporadically and were more than 1,000 years ago. In the past century there were four pandemics: 1918–1919 'Spanish' H1N1 influenza; 1957–1958 'Asian' H2N2 influenza; 1968–1969 'Hong Kong' H3N2 influenza; and 2009–2010 'Swine-origin' H1N1 influenza [6].

The emergence of pandemic (H1N1) 2009 in Mexico confirmed the need to understand the epidemiology of past pandemics in the world, which should be investigated in the future to further prevent pandemic [10].

Thus, influenza viruses continue to remain relevant pathogens, and therefore the aim of study – a comparison of the morbidity of influenza Zhytomyr region populations and Ukraine as a whole. The primary goal of this study – to analyse seasonality, cyclical recurrence of morbidity Zhytomyr region populations during from 1999 till 2011.

Materials and Methods: The information and statistical data Zhytomyr regional sanitary-epidemiological station from 1999 till 2011 (reporting forms on the annual morbidity – f.2 and monthly morbidity – f.1) were used.

Results and Discussion

Flu is inherent seasonality. In the Zhytomyr region, as in Ukraine as a whole an annual seasonal epidemic rises of influenza are recorded. Analyzing monthly dynamics of influenza population of Zhytomyr region of each calendar year from 1999 to 2011 may be noted that it has been registered in the region during all calendar years of our observation of a distinct seasonality. Increase in incidence occurs in the winter-spring period (January – March).The biggest data of influenza morbility of populations from 1999-2011, were in the 1st quarter of February (1288,86 per 100 000 population – 2008; 549,81 per 100 000 population – 2007; 395,35 per 100 000 population – 2004; 2302, 65 per 100 000 population – 2003; 637,52 per 100 000 population – 2001; 2046,18 per 100 000 population – 2000; 1946, 51 per 100 000 population – 1999). Increased morbidity associated with the output pupils and students from holidays (large crowd of people promote the spread of infection), after which increases morbidity not only in influenza, but also from ARVI.

During the summer, there is a significant decrease in morbidity of influenza. These cases are sporadic. Primarily due to the direct dependence of influenza on the temperature of the environment – the flu virus is well preserved at low temperatures, and the second – with an increase in nonspecific immunity in the population. Continuity epidemiological process also explains that in the northern hemisphere of the globe disease occurs in autumn and winter (November – March) and in the southern hemisphere – April – October. Thus there is a transfer of influenza viruses from one hemisphere to the second. There is a hypothesis about the persistence of influenza virus in the body recover from the person, and if low immunity can cause disease. People who have no immune protection against these viruses often suffer. Analyzing the dynamics of long-term (13 calendar years) influenza morbidity of Zhytomyr region populations and comparing its indicators with Ukraine as a whole can conclude that during surveillance in epidemic process of this infection proved respect to some cyclical recurrence (years of increase : 1999 (3785,6 per 100 000 population); 2003 (3556,39 per 100 000 population); 2005 (2034,57 per 100 000 population); 2009 (1595,75 per 100 000 population) and years of decrease : 2002 (438,62 per 100 000 population); 2004 (1186,41 per