МІКРОБІОЛОГІЯ, ЕПІЗООТОЛОГІЯ ТА ІНФЕКЦІЙНІ ХВОРОБИ

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ENZOOTIC CASE OF PHEASANTS ASPERGILLOSIS

У статті описується випадок хвороби фазанів на ензоотичний аспергільоз на приватній фермі м. Біла Церква Київської області у 2015 році. Хвороба супроводжувалася відставанням у рості і розвитку, виснаженням і спорадичною смертю. Характеризувалася гострим перебігом, а смертність птахів становила 80 % від загальної кількості в господарстві. Ензоотичний аспергільоз у фазанів мав такі особливості: короткий інкубаційний період (5 днів), порушення координації, діарея, в дихальних шляхах жовто-зелена плівка утворена міцелієм гриба. Мікологічні дослідження показали, що в 1 г корму міститься близько 920 тисяч КУО. Мікобіоти представлені видами A.fumigatus, які були домінуючими, а також A. ochraceus, Absidia corymbifera, Mucor spp. і Penicillium spp. Якісною реакцією з хлоридом заліза встановлена здатність ізольованого штаму A. fumigatus до продукування коєвої кислоти.

Ключові слова: A. fumigatus, A. ochraceus, Absidia corymbifera, Mucor spp. i Penicillium spp., аспергільоз, фазани.

Problem statement. Grain feed, poultry and animals infestation with toxic microscopic fungi has become worldwide spread [1]. According to the research results, microscopic fungi and their toxins affect farm animals health and productivity, cause deterioration of the physiological state and lower the organism resistance against diseases [2-5].

Analysis of recent research and publications. Contamination of feed grain and food products with microscopic fungi and mycotoxins occurs often in Ukraine [3, 5]. This is especially true for the fungi of *Aspergillus* genus, which belong to the most dangerous natural contaminants [6, 7].

Some reports reveal the negative impact of feed containing subtoxical dose of mycotoxins. According to O. Malinin et al. [5], the content of colony forming units of micromycetes of more than 40 thousand in 1g of feed resulted in gain decline and death of some animals.

Industrial poultry farms and small farms make the basis of modern poultry industry of Ukraine and the limited territory where a significant amount of livestock is farmed is their main feature which causes the danger of the various infectious diseases emergence and spreading. The biggest economic losses are caused by mycosis and poultry mycotoxicoses that can affect a significant amount of poultry in a short time period. According to scientific literature, the death from aspergillosis is 60-90% of the total livestock in the economy due to sick poultry growth and development lagging and further death [7, 8].

Despite a number of studies conducted by different researchers, further study of the aspergillosis etiology in different species of animals and birds in the conditions of small farms as well as development of effective tools and methods for its prevention and treatment are rather topical.

The purpose and objectives of the study. The research aims to determine the causes of pheasants disease and to provide practical recommendations on the prevention and elimination of the disease consequences.

A similar situation occurred in a farm of Bila Tserkva, Kyiv region. Epizootic pheasants aspergillosis were observed in pheasants of different age groups from 5- to 90 days of age. The disease had an acute course and the birds loss amounted to 80% of those set for farming. The enzootic process in pheasants aspergillosis has the following features: a short incubation period (5 days), incoordination, diarrhea, yellow-green film with fungus mycelium formation in respiratory tracts.

Dead birds corpses and infected organs were sent to the Department of Microbiology and Virology of Bila Tserkva NAU. Aspergillosis was diagnosed by the pathological anatomical changes on the ground of clinical signs and laboratory investigations.

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Methods and materials. To determine the cause of pheasants disease and death we conducted the autopsy as well as the pathological-anatomical research in the section hall of Bila Tserkva NAU. The autopsy results assumed aspergillosis, thus, we conducted mycological examination of the forage and straw bedding on order to determine the extent of their contamination with fungi, their species composition and toxity as well as made inoculation of the affected pieces medium to isolate the pathogen. For this purpose, we inoculated the feed and straw bedding samples on the Saburo and Chapek medium in Petri dishes and incubated them at a temperature of 24 and 37 °C. We defined the amount of CFU (colony forming units) of fungi in 1g of food by applying the method of serial dilutions. Since the largest share belonged to *Aspergillus* genus fungi with isolates of *Aspergillus fumigatus* domination, we used qualitative reaction with ferric chloride to determine their ability to produce kojic acid (Fig. 1).

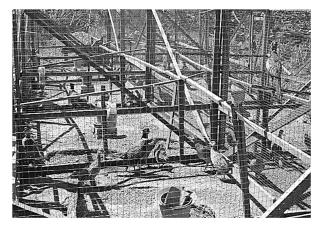




Fig. 1. Keeping pheasants at the farm.

Results and discussion. The corpses of birds aged 5- 90-day which were subject to autopsy were of poor fatness or exhausted, with the signs of lagging development. Their skin was gray covered with ruffled feathers. The orifices mucous membranes were gray or gray-reddish. The stomach contained moderate or small amount of food, mainly roughage. There has been found: sero-fibrinous pleurisy, local and diffuse pneumonia without the nodules formation, hepatyzed pulmonary tissue. The death was caused by birds stable pulmonary disease in consequence of local and diffuse pneumonia complicated by pleurisy (Fig. 2a, b).





Fig. 2. The appearance of the lung (local and diffuse pneumonia) under aspergillosis.

Disk-shaped, lamellar layers and a significant amount of red, turbid fluid have been found in the pneumatic membrane bags and intestinal serose of the abdomen. Also, hemorrhagic bowel inflammation and the liver congestion were revealed. Mycological study has found that 1g of food fed to the animals contained 920 thousand of CFU. Mycobiota was represented with *A. fumigatus*, which dominated (Fig. 3), *A. fumigatus*, *A. ochraceus*, *Rhizopus oryzae*, *Absidia corymbifera*, *Mucor spp*. and

Penicillium spp. Qualitative reaction with ferrum chloride proved the capability of *A. fumigatus* isolated strain to produce kojic acid.

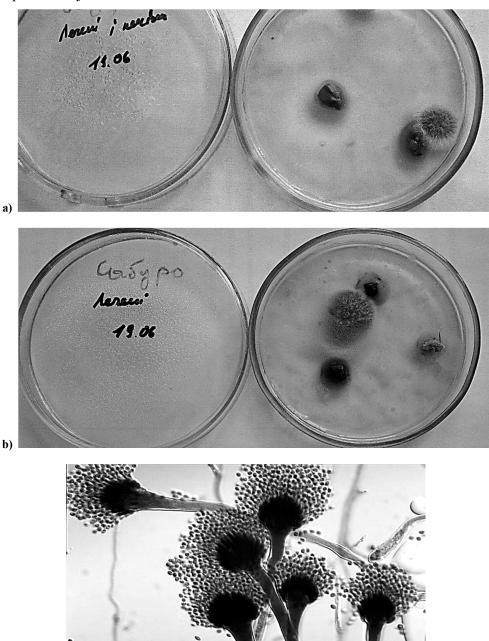


Fig. 3. The growth of *A. fumigatus* fungus colonies on Chapek (a) and Saburo (b) media (gray) in the affected parts of the lungs occulation.

Micromycetes isolated from the investigated material are potential producers of ochratoxins, aflatoxins, steryhmatocystine, aspergillic, kojic acids and other mycotoxins.

Significant contamination of feed with genus *Aspergillus* fungi caused accumulation of toxic metabolites in the feed. The fungi trace did not result in rapid death of animals. However, mycotoxins have caused inflammation of the stomach, small intestine, conditioned immune suppressive effect. They formed mucosa ulcer, which tended to increase, covering large areas. Immune suppressive effect of mycotoxins contributed to respiratory complications and pheasants died from chronic pneumonia.

Conclusions. 1. The study has found out that the cause of the pheasants disease and death was feeding heavily contaminated with micromycetes (about 920 thousand CFU in 1g) of various types, including the dominationg isolates of *A. fumigatus*, which are capable of kojic acis biosynthesis.

2. Associated action of contaminants and their metabolites in sublethal concentrations was the cause birds chronic poisoning.

LIST OF REFERENCES

- 1. Рухляда В.В. Мікологічний моніторинг зерна ячменю в різних фізико-географічних регіонах України / В.В. Рухляда, А.В. Андрійчук, О.В. Соколова // Вісник Білоцерківського ДАУ. 2006. Вип. 36. С. 149–155.
- 2. Рухляда В.В. Розповсюдження міксоміцетів на зерні вівса у різних регіонах України / В.В. Рухляда, А.В. Білан, О.В. Соколова // Вісник Білоцерківського ДАУ. –2007. Вип. 44. С. 146–150.
- 3. Білан А.В. Вивчення токсиноутворюючих властивостей *Fusarium moniliforme*, ізольованого із зернових України / А.В. Білан // Матер. V міжнар. конгр. спеціалістів вет. медицини. К.: НАУ, 2007. С. 136–138.
- 4. Vesonder R.F. Moniliformin produced by cultures of *Fusarium moniliforme* var. subglutinans isolate from swine feed / R.F. Vesonder // Mycopatologia. 1992. V. 95. P. 149–153.
- 5. Мікотоксикологічний моніторинг концентрованих кормів Лісостепу України / О. Малінін, О. Куцан, Г. Шевцова, О. Семеріна // Тваринництво України. 2003. №12. С. 26–28.
- 6. Dhama K. Fungal/mycotic diseases of poultry-diagnosis, treatment and control. / K. Dhama, S Chakraborty., A. Verma // Pak J Biol Sci. 2013, Dec 1. Vol. 16(23). P. 1626-40.
 - 7. Інфекційні хвороби тварин / Б. Ф. Бессарабов, Е. С. Воронін та ін; За ред. А. А. Сидорчука. М.: Колос, 2007. 671 с.
- 8. Liliana Scorzoni. Antifungal Therapy: New Advances in the Understanding and Treatment of Mycosis Front Microbiol. / Liliana Scorzoni, Ana C. A. de Paula e Silva, Caroline M. Marcos. 2017. Vol. 8. 36 p. Published online 2017 Jan 23. doi: 10.3389/fmicb.2017.00036

REFERENCES

- 1. Ruhlyada, V.V., Andreychuk, A.V., Sokolova, A.V. (2006) Mycological monitoring of barley in different physiographic regions of Ukraine [Mycological monitoring of barley grain in various physical and geographical regions of Ukraine], Bulletin Bila Tserkva State Agrarian University, vol. 36, pp. 149-155.
- 2. Ruhlyada, V.V., Bilan, A.V., Sokolova, A.V. (2007) Distribution Micromycetes on grain oats in different regions of Ukraine [Distribution of micromycetes on oats in different regions of Ukraine]. Bulletin Bila Tserkva State Agrarian University, vol. 44, pp. 146-150.
- 3. Bilan, A.V. (2007) Study toxicity properties *Fusarium moniliforme*, isolated from grain Ukraine [Study of toxinforming properties of Fusarium moniliforme, isolated from grains of Ukraine], Mater. V Intern. Congré. veterinary specialists. Medicine, Kyiv, NAU, pp. 136-138.
- 4. Vesonder, R.F. (1992) Moniliformin produced bu cultures of *Fusarium moniliforme* var. *subglutinans* isolate from swine feed, Mycopatologia, V. 95, pp. 149-153.
- 5. Malinin, A., Kutsan, A., Shevtsov, D. (2003) Mikotoxicity monitoring concentrated feed steppes of Ukraine [Mycotoxicological monitoring of concentrated forages of the forest-steppe of Ukrain], Animal Semerina Ukraine, №12, pp. 26-28.
- 6. Dhama K., Chakraborty S., Verma A. (2013). Fungal/mycotic diseases of poultry-diagnosis, treatment and control. Pak J Biol Sci., vol. 16(23), pp. 1626-40.
 - 7. Basarabians, B.F., Voronin, E.S. etc. Ed. A.A. Sydorchuka (2007). [Infectious diseases animals], Moskov, Kolos, 671 p.
- 8. Liliana Scorzoni, Ana C. A. de Paula e Silva, Caroline M. Marcos (2017). Antifungal Therapy: New Advances in the Understanding and Treatment of Mycosis Front Microbiol. vol. 8-36 p. Published online 2017 Jan 23. doi: 10.3389/fmicb.2017.00036

Энзоотический случай аспергиллеза фазанов

А.В. Билан, В.Н. Зоценко, А.В. Андрейчук

В статье описывается случай болезни фазанов энзоотическим аспергиллезом в частной ферме г. Белая Церковь Киевской области в 2015 году. Болезнь сопровождалась отставанием в росте и развитии, истощением и спорадической смертью. Болезнь протекала остро, а смертность птиц составляла 80 % от общего количества в хозяйстве. Энзоотический аспергиллез у фазанов имел следующие особенности: короткий инкубационный период (5 дней), нарушение координации, диарея, в дыхательных путях желто-зеленая пленка образованная мицелием гриба. Микологическое исследование показало, что в 1 г корма содержится около 920 тысяч КОЕ. Микобиота представлена видами A.fumigatus, которые были доминирующими, а также A. ochraceus, Absidia corymbifera, Mucor spp. и Penicillium spp. Качественной реакцией с хлоридом железа установлена способность изолированного штамма A. fumigatus к продуцированию коевой кислоты.

Ключевые слова: A. fumigatus, A. ochraceus, Absidia corymbifera, Mucor spp. и Penicillium spp. аспергиллез, фазаны.

Enzootic case of pheasants aspergillosis A. Bilan, V. Zotsenko, A. Andriychuk

The article described the case of pheasants malady in a private farm of Bila Tserkva area, Kyiv region observed in 2015. The illness was accompanied by growth and lagging development depletion and sporadic death. The disease had an acute course and the birds loss amounted to 80% of those set for farming. The enzootic process in pheasants aspergillosis has the following features: a short incubation period (5 days), incoordination, diarrhea, yellow-green film with fungus mycelium formation in respiratory tracts. Mycological study has found out that 1g of feed the animalsconsumed contained approximately 920 thousand of CFU. The mycobiota was represented by A.fumigatus species, which was dominant, and A. ochraceus, Absidia corymbifera, Mucor spp. and Penicillium spp. Qualitative reaction with ferric chloride revealed the capacity of the isolated strain of A. fumigatus to produce kojic acid.

Key words: A. fumigatus, A. ochraceus, Absidia corymbifera, Mucor spp. и Penicillium spp., aspergillosis, pheasants.

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