Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 120, 267—276, 2011

UDC 636.5.09:616.927.7
DOI: 10.2298/ZMSPN1120269K

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INVESTIGATION OF DISSEMINATION OF ASPERGILLOSIS IN POULTRY AND POSSIBLE CONTROL MEASURES

ABSTRACT: Fungi belonging to genus *Aspegillus* are ubiquitous saprophytic microorganisms which are, in certain circumstances, responsible for clinical infections of respiratory tract in all poultry, particularly in young birds. In case of a lung form, *Aspergillus fumigatus*, *A. niger* and *A. glaucus* are the most frequently isolated fungi. In general, poultry is constantly exposed to these fungi in its environment. Predisposing factors, such as long exposition and highly contaminated environment and litter, high humidity in poultry houses, poor ventilation, malnutrition and stress, all contribute to clinical aspergillosis. Some geographic and seasonal regularities are observed in relation to the distribution of disease outbreaks. In this sense, cases of aspergillosis in our country were more frequently noted in wild areas located northern from the rivers Sava and Danube. Influence of some factors on the outbreak and spreading, as well as predominant clinical features of aspergillosis in poultry were investigated in this paper. Possible prophylactic and intervention measures were discussed. The occurrence of *Aspergillus sp.* in poultry was analyzed according to the clinical and laboratory investigations performed during the two selected years, 2000 and 2010. Widespread aspergillosis was noted in poultry flocks of different age, both in young and adult birds. During the years 2000 and 2010, acute aspergillosis was found in 12 and 16 commercial flocks of chickens and turkeys, respectively. Ocular infection with *Aspergillus* was determined in 10 day old broilers from two flocks. *Aspergillus sp.* was isolated from unhatched eggs (6.86%), litter (23.07%), environmental (36.17%) and hatchery swabs (3.85%). Besides the appropriate antifungal therapy, enforcement of proper sanitary-hygiene measures on poultry farms and hatcheries, as well as microbiological control of feed are considered essential for an efficient control of infection and its spreading.

KEY WORDS: avian aspergillosis, humidity, environment, temperature

INTRODUCTION

Aspergillosis is a fungal disease of all poultry species, particularly of young birds. Most frequently it occurs in turkey poults, chicks, ducklings and goslings (K u n k l e, 2003; B e y t u t et al., 2004). *Aspergillus fumigatus* is considered to be the most pathogenic and is the most frequent isolate from pathologic lesions, while others like *A. niger*, *A. flavus*, *A. terreus* and *A. glaucus*

can induce the disease, too. The fungal spores are ubiquitous in nature. Exposure of poultry to fungi or spores occurs after the introduction of contaminated litter and feed. Early infection is possible in hatcheries if fungal contamination occurs.

Certain infectious diseases may contribute to aspergillosis, e.g. infectious bronchitis, coryza, chronic respiratory disease, laryngotracheitis, Newcastle disease and fowlpox. It has been speculated that extremly dry air and dust can cause the infection with aspergillus because they dry out the respiratory mucoses and protective effect of mucus is absent (K r i s t e n s e n and W a t h e s , 2000).

Some geographic and seasonal regularities are observed in relation to the distribution of aspergillosis outbreaks. Incidence of the disease decreases on poultry farms with stringent hygiene and good nutrition menagement.

Influence of certain factors on the outbreak and spreading, as well as clinical features of aspergillosis, were investigated in this paper. The possibility to control the disease by introduction of prophylactic and intervention measures was discussed.

MATERIAL AND METHODS

The presence of *Aspergillus* spp. on poultry farms was analyzed from data collected after the clinical and laboratory investigations performed during the two selected years, 2000 and 2010.

Poultry flocks were clinically observed and postmortem examination of chickens and turkeys of different age was carried out. If aspergillosis was detected in the findings, the attacked tissues were taken for microbiological investigations. Having been processed first, tissue samples were cultured on solid media using standard methods. Mycological investigations were done in accredited laboratory at the Veterinary Institute Novi Sad.

Litter taken directly from different surfaces in poultry houses and swabs was cultured on solid media. Unhatched eggs, hatchery waste and environmental swabs taken from different surfaces in hatcheries were sampled in order to determine the presence of fungi and its frequency, as well as for the comparison of mycological contamination with the environmental conditions.

The influence of different factors on the occurrence, spreading and source of aspergillosis was investigated using data from the official report of the Republic Hydrometeorological Service of Serbia, regarding the outer temperature and rainfalls in 2010, for the purpose of comparison with the control period from 1961 to 1990.

RESULTS AND DISCUSSION

The spreadout of aspergillosis was noted in poultry of different age, from very young birds to adult ones. It is well known that the disease can occur in

other young and adult poultry species, including poults, gooslings, ducklings, swans, wild and pet birds, particularly if kept in intensive manner. While resistant and healthy poultry can overcome the infection with high number of *Aspergillus*, week and young birds generally become ill easily. It is experimentally demonstrated that chickens up to three days of age are the most susceptible to the infection, while older are more resistant. I s l a m et al. (2009) found significant differences in morbidity (up to 70%) and mortality rate depending on age and category; aspergillosis was most frequently detected in cockereles (9.03%), then in broiler chickens (5.48%) and in laying hens (1.92%). The disease was clinically present in broilers at the earliest age (13 days) and in layers and cockereles at the oldest age (76 weeks). In epizootiological investigation S a j i d et al. (2006) found the majority of sick flocks at the age of 14 days.

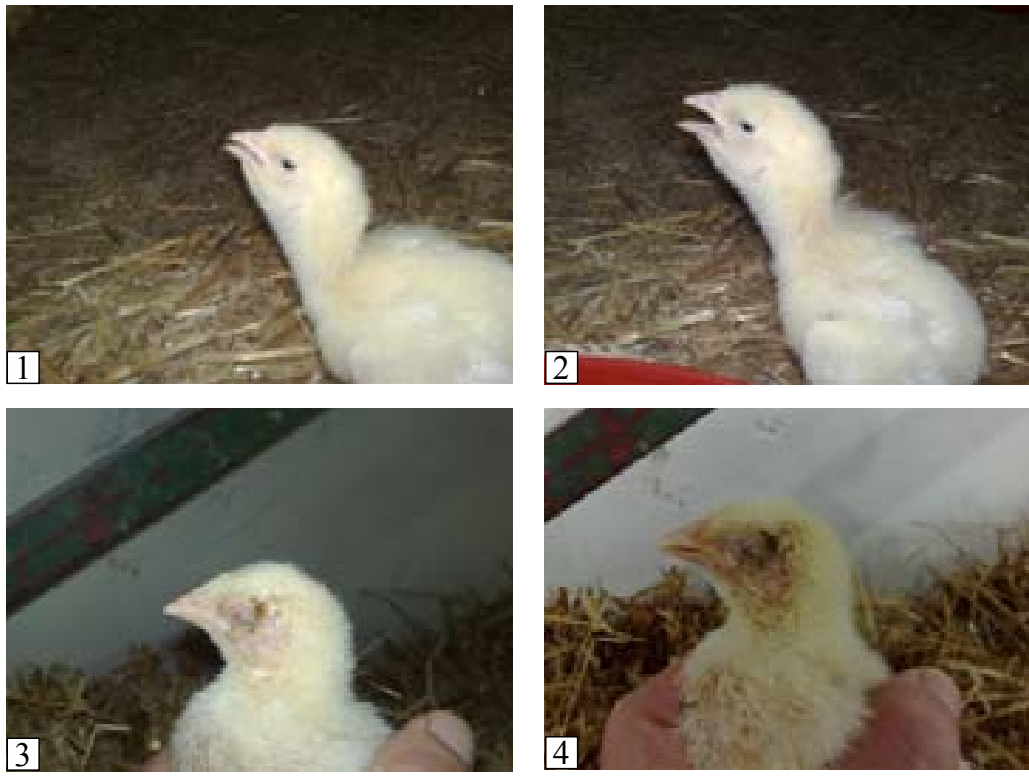
Aspergillosis apears in two clinical forms: 1) acute form with high morbidity and mortality rates, the disease persists from only few days to two weeks, and 2) subacute and chronic form in adult poultry, disease being persistant several weeks or rarely several months.

During the years 2000 and 2010, acute aspergillosis was detected in 12 and 16 commercial flocks of chickens and turkeys, respectively. In 2010, the disease was acute with high morbidity and mortality within only few days. Numerous flocks suferred detrimental losses in a short period of time and were destroyed because their further raise was economically unjustified. Clinical symptoms during the acute source of infection are described in literature, including depression, inapetence, thirst and hyperventilation with dyspnoe (A k a n et al., 2001; I s l a m et al., 2009; S t o u t e et al., 2009). Chickens have ruffled feathers; they drowse, get weaker and, in complicated cases of dyspnoe, pathologic wheese is observed, which differs from other respiratory diseases. Besides the symptoms previously mentioned, the investigation of mass pneumomycoses discovered one distinctive finding which was grupping of the chickens toward the source of fresh air ("hunger for oxygen"; Figures 1 and 2).

Besides respiratory form, infection of eyes can also occur (A k a n et al., 2001). In that case protruding eye lids are observed because of formation of yellowish-cheesy small pellets around the membrane nicticans with central ulceration (Figures 3 and 4). In our investigations, ocular infection with *Aspergillus* was determined in two broiler flocks, at the age of 10 days.

In subacute and chronic infection, only one or several individuals in flock became ill. Clinical signs of depression, inapetence, ruffled feathers and emaciation are common, sometimes chronic bronchopneumonia, too (M a r t i n et al., 2007; I s l a m et al., 2009). during the year 2010, subacute and chronic aspergillosis was not detected.

In the investigated period, acute aspergillosis was clinically noted. Postmortem findings included nodules – aspergillus granuloma, oval or round shaped, single or in conglomerate, size of a pin head to pea, located on air sacs, lungs and on visceral serosae of abdominal cavity, liver and intestines (Figures 5, 6, 7 and 8). Granulomas, oval or round, single or in group of conglomerate, are typical section finding in *Aspergillus* infected birds. They are located in tho-



Slika 1 i 2. Oboleli pilići od aspergiloze – izražena dispnoja.
Slika 3 i 4. Očni oblik aspergiloze – žućkaste siraste kuglice oko membrane niktikans.

racic and abdominal cavity and on the liver surface. Their appearance resembles the one found in avian tubercullosis, but with radial hyphae nets surrounded by reactive zone like granulation tissue. Generally, nodules are present in almost all tissues, even in eyes and brain (Throne Steinlage et al., 2002; Mukaratirwa, 2006; Martin et al., 2007; Cacciuttolo et al., 2009; Islam et al., 2009; Stoute et al., 2009).

Clinical signs and pathologic findings are not sufficient for a diagnose, so laboratory confirmation by isolation of *Aspergillus sp.* is necessary. The spores germinate well in laboratory conditions, on standard media and at room temperature, producing green to green-blue colored colonies that become darker, even black over time (Figures 9 and 10). In order for the disease to be diagnosed other respiratory agents need to be excluded, such as infectious bronchitis, coryza, chronic respiratory disease and Newcastle disease, infectious laryngitis, tuberculosis and fowlpox.

In intensive poultry keeping, there are numerous sources of this important fungal infection. Feed, particularly mashed, can be contaminated with high number of fungi and molds (Akan et al., 2001; Nešić et al., 2005; Škrinjar et al., 2009). According to the Regulation of microbiological quality of feed, complete diets for young and adult poultry may contain not more then 50000 and 300000 colonies of molds, respectively. Based on our knowledge, findings




Slika 5-8. Oboleli ćurići od aspergiloze; patoanatomski nalaz: diseminirani čvorići-aspergilusni granulomi na plućima i zamućenim vazdušnim kesama (slika 5), i po serozama organa u abdominalnoj duplji (slika 6,7,8)


Slika 9 i 10. Rast kolonija Aspergillus flavus na Sabour-o agaru

of microbiologically inadequate complete diets with *Aspergillus* contamination are not rare (data not presented here).

The infection in poultry may occur if litter, environment or hatcheries are contaminated. Fertile eggs and embryos can be contaminated before or during the incubation, and *Aspergillus sp.* is found in tissues of unhatched eggs and hatchery waste (J a c o b s e n et al., 2010). Warm and humid air in hatchery provides ideal conditions for the survival of *Aspergillus*. Infected embryos die

between 15th and 18th incubation day, which may cause a dicrease in hatchability of up to 30%. Dust containing more than 800 colonies per gram leads to prospective embryo infection (K o z i ć, 1967). Eggshell can be contaminated in case of inadequate collection and storage of eggs. The spores are most frequently isolated from alanto-chorion liquid, producing green colonies up to 2 cm in size. During the years 2000 and 2010, *Aspergillus sp.* colonies were often detected in unhatched eggs and swabs taken from the hatchery (Table 1). In order to prevent the contamination of embryos and chickens at hatch, different sanitation programs are applied (I v a n o v, 2008). The sanitation programs are designed in such manner that the number of fungi and other potential pathogens is maintained at an acceptable level. However, their total elimination is not realistic. Lately, some reports have brought the attention to possible infection of reproductive tissues of hens and concomitant disease (*Egg Borne Aspergillosis* or congenital aspergillosis). It is demonstrated that intravenous and inhalation infection is possible (F e m e n i a et al., 2007). Still, under natural conditions, infection is predominantly induced aerogenically and orally.

Using inadequate litter increases the possibility of infection at early age. Higher number of fungi, longer exposition, particularly in combination with stressing procedures, like vaccination, beek trimming etc., all contribute to aspergillosis. Different types of litter may be contaminated unequally. S a j i d et al. (2006) found significantly higher rate of infected eggs in flocks kept on sawdust (67.74%) in compairison to rice hulls (32.26%). On the other hand, I s l a m et al. (2009) could not clearly relate the incidence of aspergillosis to the type of litter used. In our investigations, mycological control of litter and swabs taken from surfaces in poultry houses points to oversights during sanitation and disinfection (Table 1). With respect to year 2000, the total number of samples and the number of mycologically inadequate samples was nominally higher in 2010. Raising poultry under higher fungal contamination increases the risk of aspergillosis, especially in immune compromised or sick birds. The influence of particular outer and ambiental factors to fungal survival are numerous (D e B e y et al., 1995; G i g l i et al., 2005; K a r w o w s k a, 2005; N i c h i t a and T i r z i u, 2008). The spores of *Aspergillus* germinate better when the air is dry. *Aspergillus sp.* is often isolated from dust, and since the largest proportion of dust particles is inhalable, dust is considered to be the predisposing factor for aspergillosis outbreak (D e B e y et al., 1995). The type of ventilation and air humidity are also relevant (D e B e y et al., 1995; G i g l i et al., 2005; S a j i d et al., 2006) as they can impact the concentration of *Aspergillus* in the poultry house (N i c h i t a and T i r z i u, 2008).

By using available data on meteorological conditions in the region, this paper aims at establishing the connection between clinical aspergillosis and overall laboratory findings, as well as the climatic factors. The official report of Hydrometeorological Service of the Republic Serbia for the year 2010 show extremely warm percentile distribution. During the summer of 2010 maximal daily temperatures were often above 30^0C, and the total number of tropical days was higher then the usual in most parts of Serbia, except in mountain regions.

Tab. 1 – Contamination of unhatched eggs, litter, swabs taken from the poultry houses and hatcheries by *Aspergillus sp.*

Sample type	Year 2000		Year 2010	
	Total number of samples	Number and % of positive samples	Total number of samples	Number and % of positive samples
Unhatched eggs	481	33 – 6.86	617	62 – 10.05
Litter	39	9 – 23.07	42	15 – 35.71
Swabs from houses	94	34 – 36.17	111	39 – 36.14
Hatchery swabs	182	7 – 3.85	268	15 – 5.60

The Hydrometeorological reports also show rainfalls of totally 110 to 150% of the average values (taken from the period between 1961 and 1990) in most parts of Serbia, and the percentile distribution was rainy, very rainy and extremely rainy. The rainfalls were extremely rainy in the northern regions. During the selected years (2000 and 2010) average outer temperatures were high throughout the whole year. The rainfalls, watery alluvium and relative humidity of air, were significantly higher than usual. In order to survive and reproduce, fungi need certain humidity and temperature level which is around and higher than 37ºC (De Bey et al., 1995; Karwowska, 2005; Gigli et al., 2005; Sajid et al., 2006; Chate and Bhivgade, 2010).

The distribution of the aspergillosis outbreaks is influenced by some geographic and seasonal differences, so it is more frequent in areas with high outer temperature and rainfalls (De Bey et al., 1995; Karwowska, 2005; Madsen, 2006; Sajid et al., 2006). On the other hand, during winter, due to inadequate ambiental conditions and the fact that sometimes it is impossible to provide optimal ambient for poultry, aspergillosis incidence increases. Some daily aberrance in the number of *Aspergillus* in poultry houses was determined by Nayak et al. (1998). In our country, aspegillosis outbreaks are more frequent in areas northern from the rivers Sava and Danube.

Microflora in poultry houses and farms in general, in our case the "population" of *Aspergillus sp.,* contributes to the emission of biosol into atmosphere, especially during the warmer months (Karwowska, 2005). The concentration of unwanted and potentially harmful gasses, dust, bacteria and fungi in poultry house changes depending on the age of poultry, particularly in broiler chickens (Vučemilo et al., 2007). The airpollution impacts the health and productivity of poultry (Nešić et al., 2005). Also, certain species of fungi have toxinogenic and allergenic features so they can disturb the health of people, either directly, in case of farmers, or in case of the rest of the population, indirectly, after emission into atmosphere (Škrinjar et al., 2009; Chate and Bhivgade, 2010). It seems to be necessary to create and implement standards for adequate hygiene, epizootiological and epidemiological conditions on farms and their environment.

CONCLUSIONS

1. The global warming and high relative humidity induced increase of incidence of some diseases, including aspergillosis.

2. Poultry is constantly exposed to *Aspergillus sp.* in its environment, so high contamination and long exposal contribute to clinical aspergillosis.

3. The success of therapy basically depends on the source of disease and degree of dissemination of process. In acute cases, particularly in very young chickens, it would be wrong to rely on antifungal therapy solely. Implementation of sanitary-hygiene measures in poultry houses and hatcheries, as well as microbiological control of feed, is essential for prevention of significant losses.

REFERENCES

Akan, M., Hazrolu, R., lhan, Z., Sareyyüpolu, B., Tunca, R. (2001): *A case of Aspergillosis in a broiler breeder flock*. Avian Diseases 46(2): 497–501.

Beytut, E., Özcan, K., Erginsoy, S. (2004): *Immunohistochemical detection of fungal elements in the tissues of goslings with pulmonary and systemic Aspergillosis*. Acta Veterinaria Hungarica 52(1): 71–84.

Cacciuttolo, E., Rossi, G., Nardoni, S., Legrottaglie, R., Mani, P. (2009): *Anatomopathological aspects of avian aspergillosis*. Vet Res Commun 33: 521–527.

Chate, D. B. and Bhivgade, S. W. (2010): *Fungal disease incidence inside poultry farm at AUSA*. International Referred Research Journal II (15): 10-11.

DeBey, M. C., Trampel, D. W., Richard, J. L., Bundy, D. S., Hoffman, L. J., Meyer, V. M., Cox, D. F. (1995): *Effect of environmental variables in turkey confinement houses on airborne Aspergillus and mycoflora composition*. Poultry Science 74: 463-471.

Femenia, F., Fontaine, J., Lair-Fulleringer, S., Berkova, N., Huet, D., Towanou, N., Rakotovao, F., Granet, O., Le Loc'h, G., Arné, P., Guillot, J. (2007): *Clinical, mycological and pathological findings in turkeys experimentally infected by Aspergillus fumigatus*. Avian Pathology 36(3): 213–219.

Gigli, A. C., Baracho, M. S., Nääs, I. A., Silva, R. A., Zago, R., Dall'Anese, F. P. (2005): *Diagnosis and evaluation of fungi presence in the air of two different ventilation systems for broiler houses*. Revista Brasileira de Ciência Avicola 7(4): 205-208.

Ivanov, I. (2008): *Disinfection of eggs contaminated with some fungi and moulds*. Trakia Journal of Sciences, 6(Suppl.1): 98-101.

Islam, M. N., Rashid, S. M., Juli, M. S., Rima, U. K., Khatun, M. (2009): *Pneumomycosis in chickens: Clinical, pathological and therapeutical investigation*. Int. J. Sustain. Crop Prod. 4(3): 16-21.

Jacobsen, I., Große, K., Slesiona, S., Hube, B., Berndt, A., Brock, M. (2010): *Embryonated eggs as an alternative infection model to investigate Aspergillus fumigatus virulence*. Infection and Immunity 78(7): 2995–3006.

Karwowska, E. (2005): *Microbiological air contamination in farming environment.* Polish Journal of Environmental Studies 14(4): 445-449.

Kozić, (1967): *Mikotične bolesti*. In: Kozić, LJ. and Puhač, I. Eds., Gajenje i zdravstvena zaštita živine, Odbor za izdavačku delatnost Saveza veterinara i veterinarskih tehničara SFRJ, Beograd, 329-331.

Kristensen, H. H. and Wathes, C. M. (2000): *Ammonia and poultry welfare: a review.* World's Poultry Science Journal, 56: 235-245.

Kunkle, R. A. (2003): *Fungal infections. In*: Saif YM Ed., Diseases of poultry Ed. 11, Iowa State University Press, Ames, 883-902.

Madsen, A. M. (2006): *Exposure to airborne microbial components in autumn and spring during work at Danish biofuel plants*. Ann. Occup. Hyg. 50(8): 821–831.

Martin, M. P., Bouck, K. P., Helm, J., Dykstra, M. J., Wages, D. P., Barnes, H. J. (2007): *Disseminated Aspergillus flavus infection in broiler breeder pullets*. Avian Diseases 51(2): 626-631.

Mukaratirwa, S. (2006): *Outbreak of disseminated zygomycosis and concomitant pulmonary Aspergillosis in breeder layer cockerels*. J. Vet. Med. B 53: 51–53.

Nayak, B. K., Nanda, A., Behera, N. (1998): *Airborne fungal spores in an industrial area; seasonal and diurnal periodicity*. Aerobiologia 14: 59-67.

Nešić, K., Mašić, Z., Sinovec, Z. (2005): *Trovanja životinja izazvana sekundarnim metabolitima plesni*. Veterinarski glasnik 59(1-2): 41-57.

Nichita, I. and Tirziu, E. (2008): *Investigations on airborne fungi in poultry houses*. Lucrari stiintifice Medicina veterinara XLI: 932-935.

Pravilnik o kvalitetu hrane za životinje (2010): Službeni glasnik Republike Srbije 4.

Republički hidrometeorološki zavod Srbije (2010): www.hidmet.gov.rs

Sajid, M. A., Khan, I. A., Rauf, U. (2006): *Aspergillus fumigatus in commercial poultry flocks, a serious threat to poultry industry in Pakistan*. J. Anim. Pl. Sci. 16(3-4): 79-81.

Stoute, S. T., Bickford, A. A., Walker, R. L., Charlton, B. R. (2009): *Mycotic pododermatitis and mycotic pneumonia in commercial turkey poults in northern California*. J Vet Diagn Invest 21: 554–557.

Škrinjar, M., Könyves, T., Ač, M. (2009): *Frequency of Aspergillus fumigatus fres. – A toxigenic and allergenic fungal species in milking cows feeds throughout one research year*. Zbornik Matice srpske za prirodne nauke 116: 101-112.

Throne Steinlage, S. J., Sander, J. E., Brown,T. P., Lobsinger, C. M., Thayer, S. G., Martinez, A. (2002): *Disseminated mycosis in layer cockerels and pullets*. Avian Diseases 47(1): 229–233.

Vučemilo, M., Matković, K., Vinković, B., Jakšić, S., Granić, K., Mas, N. (2007): *The effect of animal age on air pollutant concentration in a broiler house*. Czech J. Anim. Sci. 52(6): 170–174.

ИСТРАЖИВАЊА РАШИРЕНОСТИ АСПЕРГИЛОЗЕ КОД ЖИВИНЕ И МЕРЕ КОНТРОЛЕ

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Резиме

Гљивице из рода *Aspegillus* су убиквитарни сапрофити који у одређеним околностима могу да изазову респираторне инфекције свих врста живине, а нарочито младих категорија. *Aspergillus fumigatus*, *A. niger* и *A. glaucus* су најчешће изоловане врсте у форми плућне аспергилозе живине. Живина је у мањој или већој мери континуирано изложена овој врсти плесни у животном окружењу, па развоју клинички манифестне аспергилозе доприносе висока експозиција, тј. јака контаминираност плеснима, висока влажност у објектима, слаба вентилација, неухрањеност и стрес. У раду је испитиван утицај појединих фактора на појаву, раширеност и клиничку форму аспергилозе код живине. Разматрана је могућност контроле болести увођењем профилактичких мера. Присуство *Aspergillus* sp. код живине је анализирано на основу резултата клиничких и лабораторијских испитивања извршених у току 2000. и 2010. године. Раширеност аспергилозе је констатована код живине у различитом узрасту, од младих категорија па до узраста постигнуте технолошке старости. У току 2010. године аспергилоза живине, код пилића и ћурића регистрована је у акутном току у укупно 16 јата, нешто више него у току 2000. године, када је утврђено 12 клинички оболелих комерцијалних јата пилића и ћурића. Регистрован је случај инфекције на очима у два јата бројлерских пилића у узрасту од 10 дана. Миколошким контролама узорака неизлежених пилића, простирке, брисева са површина из објеката и инкубатора, *Aspergillus* sp. је изолован у распону од 3,85% до 36,14 %.

За контролу ширења инфекције од пресудног је значаја примена санитарно-хигијенских мера у објектима за смештај живине и инкубаторским станицама, као и контрола микробиолошког квалитета хране.