INVESTIGATION OF DISSEMINATION OF ASPERGILLOSIS IN POULTRY AND POSSIBLE CONTROL MEASURES

ABSTRACT: Fungi belonging to genus Aspergillus 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 (Kunkle, 2003; Beyer t u 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 extremely dry air and dust can cause the infection with aspergillus because they dry out the respiratory mucoses and protective effect of mucus is absent (Kristensen and Wathes, 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 management. 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. Islam 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 cockerel chickens (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 cockerel chickens at the oldest age (76 weeks). In epizootiological investigation Sajid et al. (2006) found the majority of sick flocks at the age of 14 days.

Aspergillosis appears 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 persistent 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 suffered 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 (Akan et al., 2001; Islam et al., 2009; Stout et al., 2009). Chickens have ruffled feathers; they drowse, get weaker and, in complicated cases of dyspnoe, pathologic wheeze is observed, which differs from other respiratory diseases. Besides the symptoms previously mentioned, the investigation of mass pneumomycoses discovered one distinctive finding which was gruing 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 (Akan 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 (Martin et al., 2007; Islam 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-
racic and abdominal cavity and on the liver surface. Their appearance resembles the one found in avian tuberculosis, 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; Škriňar 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
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 (Jacobse 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 decrease in hatchability of up to 30%. Dust containing more than 800 colonies per gram leads to prospective embryo infection (Kozic, 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 (Ivanov, 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 (Femenia 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. Sajid et al. (2006) found significantly higher rate of infected eggs in flocks kept on sawdust (67.74%) in comparison to rice hulls (32.26%). On the other hand, Islam 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 (De Bey et al., 1995; Gigli et al., 2005; Karwowska, 2005; Nichita and Tirziu, 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 (De Bey et al., 1995). The type of ventilation and air humidity are also relevant (De Bey et al., 1995; Gligl et al., 2005; Sajid et al., 2006) as they can impact the concentration of Aspergillus in the poultry house (Nichita and Tirziu, 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°C, and the total number of tropical days was higher then the usual in most parts of Serbia, except in mountain regions.
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 37°C (De Bey et al., 1995; Karwowska, 2005; Gigli et al., 2005; Sajid et al., 2006; Chat e and Bhiv gade, 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 (Vuce milo et al., 2007). The airpollution impacts the health and productivity of poultry (Nesic 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 (Skrinjar et al., 2009; Chat e and Bhiv gade, 2010). It seems to be necessary to create and implement standards for adequate hygiene, epizootiological and epidemiological conditions on farms and their environment.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Year 2000</th>
<th></th>
<th>Year 2010</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number of samples</td>
<td>Number and % of positive samples</td>
<td>Total number of samples</td>
<td>Number and % of positive samples</td>
</tr>
<tr>
<td>Unhatched eggs</td>
<td>481</td>
<td>33 – 6.86</td>
<td>617</td>
<td>62 – 10.05</td>
</tr>
<tr>
<td>Litter</td>
<td>39</td>
<td>9 – 23.07</td>
<td>42</td>
<td>15 – 35.71</td>
</tr>
<tr>
<td>Swabs from houses</td>
<td>94</td>
<td>34 – 36.17</td>
<td>111</td>
<td>39 – 36.14</td>
</tr>
<tr>
<td>Hatchery swabs</td>
<td>182</td>
<td>7 – 3.85</td>
<td>268</td>
<td>15 – 5.60</td>
</tr>
</tbody>
</table>
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 exposure contribute to clinical aspergillosis.

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

REFERENCES


ИСТРАЖИВАЊА РАШИРЕНОСТИ АСПЕРГИЛОЗЕ КОД ЖИВИНЕ И МЕРЕ КОНТРОЛЕ

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Резиме

Гљивице из рода Aspergillus 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 вентиляција, неухрањеност и стрес. У раду је испитиван утицај поједињих 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 изврших у току 2000. и 2010. године. Раширеност аспергилозе је констатована код живине у различитом узрасту, од младих категорија па до узрasta постигнуте технологске 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 бројлера код пилића у узрасту од 10 дана. Миколошким контролама узорака неизлежених пилића, простирке, брисева са површине 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.