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INTESTINAL PARASITES OF POULTRY IN INTENSIVE FARMING WITH SPECIAL EMPHASIS ON *BLASTOCYSTIS SP.*

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SUMMARY: The prevalence of most parasitic diseases of poultry is significantly reduced in commercial farming systems as a result of improved conditions of keeping, hygiene and management. However, parasitic diseases are still of great importance in intensive farming systems on deep litter or in "free range" breeding. A large number of parasites can cause disruptions in productivity, such as reduced weight gain, poor feed conversion, reduced egg production and even fatal outcome in severe infestation. In the practical part of the survey we examined poultry feces of different categories and age, from different poultry farms. Research has proven the presence of intestinal parasites Heterakis gallinarum, Ascaridia galli, Eimeria spp. and Blastocystis spp. in poultry fecal material. About all of this parasites, except Blastocystis we know more or less everything. For the reason Blastocystis is an emerging parasite in poultry production our discussion is mainly based on this zoonotic protozoa.

Key words: *intestinal parasites, poultry, Heterakis gallinarum, Blastocystis.*

INTRODUCTION

Poultry production/farming is spread throughout the world, both in extensive, and in intensive form. Unlike some products of animal origin, production, marketing and consumption of poultry products is related to less social and religious taboos. These factors have significantly influenced that poultry products become the most important source of protein for human nutrition worldwide. Expansion of poultry production, compared to other food industries of animal origin, suitable for easy industrialization, rapid turnover, low cost production and establishing an effective prophylaxis of diseases. Important factors in the continued development of poultry production in many countries include: market acceptability and attractiveness of poultry meat, competitive

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price and relative ease of mastering and application of new technologies, such as health-care systems.

There are basically two systems of poultry farming: the modern commercialized-intensive systems and traditional-extensive systems. Extensive poultry farming systems are characterized by low inputs in the field of breeding, nutrition and disease control and low outputs. Intensive system includes high inputs but also high outputs. Closing the chain of production, improving methods of sanitation and disinfection, pest control, and production under the principle "all in all-out", as well as prophylactic use of vaccines and antibiotics have significantly reduced the occurrence of disease in the modern poultry industry. However, widespread and uncontrolled use of antibiotics has led to the emergence of microorganisms resistant to many antibacterial and antiparasitic drugs. The development of resistant pathogens, like E. coli, Salmonella, Eimeria spp. etc. leads to significant problems in the commercial poultry sector. Particular importance is given to zoonotic diseases such as salmonellosis and campylobacteriosis. Besides all this concern about drug residues in poultry products is increasing among consumers.

The prevalence of most parasitic diseases of poultry was significantly reduced in commercial farming systems as a result of improved housing conditions, hygiene and management. However, parasitic diseases are still of great importance in deep litter systems, free-range and organic production systems. In poultry, a large number of parasites can cause disruptions in productivity, such as reduced weight gain, poor feed conversion, reduced egg production and even death in severe infestations. In addition, the parasites can greatly reduce the organism's resistance to various diseases, reduce the immune response after vaccination, and lead to exacerbation of pre-existing pathology. If we take into account the fact that in our country poultry farming is done mainly in systems with deep litter, or due to economic reasons little importance is given to biosecurity measures, antibiotics and other drugs are used unrestrained, there are all the conditions present for development, reproduction and harmful effects of parasites, even in intensive poultry farming.

Aim of the research was to compare two different industrialized poultry production systems from the aspect of presence or absence of intestinal parasites. One of our goals was also to make an insight in the prevalence of Blastocystis sp. in poultry, and try to explain some related epidemiological and clinical aspects.

MATERIAL AND METHODS

We conducted a cross-sectional prevalence study of intestinal parasites in various poultry production systems from four farms on the territory of Vojvodina, during spring-autumn 2010. The study included farms with deep litter production systems and a farm with 2/3 floor grids in the houses.

In the first stage we compared two farms, one with deep-litter production system and one with 2/3 floor grids. We collected 20-20 fecal samples from both farms. A hazelnut sized homogenized fecal samles were suspended in 20-30 ml technical glycerine in test tubes and centrifugated at 1500 rpm for 4-6 minutes. After completing the spin one drop of supernatant was transferred with a plastic tube on glass slides. Prepared samples were then observed at low and high magnification under a light microscope.

In the second stage we collected 12 fecal samples from poultry of different categories and ages, from all four farms. A collective sample consisted of 50 g of feces,

which was uniformly collected from a number of places within one or more objects. As a diagnostic procedure we used the method of direct microscopic examination of native preparations, now without flotation-concentration. Fecal samples were well mixed and homogenized. We poured into the test tubes about 20-30 ml of normal saline and then transferred into them a small amount of homogenized feces and then we mixed it using a wooden stick. A drop of the sample thus prepared was placed on glass slides using a plastic tube. A number of slides were prepared without staining, and some of the preparations we stained by Giemsa and finally covered with glass cover slips. In this way, native preparations were obtained which were then observed under light microscopy at low and then high magnification.

RESULTS AND DISCUSSION

Preparations that were prepared by flotation concentration revealed that the incidence of intestinal parasites is largely expressed in a system with deep litter, while our study did not prove the presence of any forms of intestinal parasites in the system with the grid floor. From 20 samples of feces from the system with deep litter in 6 cases there was a smaller or larger number of nematode eggs Heterakis gallinarum, while in one sample besides Heterakis gallinarum eggs we also found Ascaridia galli eggs. Our null hypothesis, that there are no differences between the two production systems from the aspect of intestinal parasites appearance, is rejected with 99% of confidence based on the conducted t-test (t_0=5,824, $t_{(40,99)}$=3,551; t_0>t). In the second stage, light microscopy revealed that out of 12 examined, eight samples were positive for some type of intestinal parasites (Table 1).

Table 1. The results of direct light microscopy (stage two of the research)

Sample no.	Category	Age	Farming system	Parasites found
1	Layer parent stock (in lay)	37 weeks	deep litter	Blastocystis sp., Heterakis gallinarum
2	Layer parent stock (in lay)	87 weeks	deep litter	Blastocystis sp.
3	Broiler parent stock (in lay)	77 weeks	deep litter	-
4	Pullets (in rearing)	10 weeks	deep litter	-
5	Pullets (in rearing)	4 weeks	deep litter	Eimeria spp.
6	Broilers	7 weeks	deep litter	Blastocystis sp.
7	Broiler parent stock (in lay)	28 weeks	2/3 floor grid	Blastocystis sp.
8	Broiler parent stock (in lay)	60 weeks	2/3 floor grid	Blastocystis sp.
9	Broilers	5 days	deep litter	-
10	Pullets (in rearing)	6 weeks	deep litter	-
11	Pullets (in rearing)	17 weeks	deep litter	Blastocystis sp.
12	Commercial laying hens	33 weeks	deep litter	Blastocystis sp.

One sample was positive for Heterakis gallinarum and Eimeria spp., while as many as 7 samples were positive for *Blastocystis*. Microscopy demonstrated a high prevalence of Blastocystis in poultry from 3 out of 4 farms. Age, production category and farming system had no effect on the prevalence of *Blastocystis* in poultry. *Blastocystis* is equally

present in younger and older categories of poultry, except that the prevalence in laying poultry (over 18 weeks of age) was above 80% (5 of 6 samples were positive), whereas in rearing it was much lower, only about 30% (2 of 6 samples were positive). *Blastocystis* was present also in heavy and light provenance poultry, in both housing systems. Number of *Blastocystis* organisms in fecal material was approximately the same on all three farms, or for all categories of poultry, and amounted to approx. 4-10 per field, at magnification of 400 times. The cells varied in their size from approximately 5-50 μm. The cells were generally spherical or oval shaped. The cytoplasm of most cells was noticeable as a thin zone around the central vacuole. By light microscopy organelles were poorly visible.

Despite the ubiquity of the genus *Blastocystis* in the intestinal tract of humans and range of animals, very little is known about this organism (Zierdt, 1991; Boreham and Stenzel, 1993). Information about the taxonomy, life cycle, transmission, host range and pathogenicity of *Blastocystis* is not definitive. Most of the presently accepted data have derived from human isolates of Blastocystis hominis (Lee and Stenzel, 1999).

Blastocystis hominis was first reported as a yeast in human faecal samples. Following a series of physiological and morphological comparisons carried out in the 1960s *Blastocystis* was placed among the protozoans, but only recently it was classified as a protozoa after an analysis of small-subunit rRNA sequences placed it firmly as a member of the Stramenopiles along with other organismas such as diatoms, brown algae, slime nets, and water moulds (Silberman et al., 1996). For over 80 years the general opinion was that *Blastocystis hominis* is an intestinal parasite restricted to humans and other primates (Zierdt et al., 1988), but in the past 20 years *Blastocystis hominis* like organisms have been found in variety of animals such as mammals, birds, reptiles, amphibians and occasionally in insects. These include *Blastocystis galli* from chickens (Belova and Kostenko, 1990), *Blastocystis anatis* from ducks (Belova, 1991) and *Blastocystis anseri* from geese (Belova, 1992). Although molecular studies on *Blastocystis* isolates from humans and animals showed that the parasite is genetically polymorphic, most of the isolates from humans and animals are identical or very close to each other based on some genetic indices (Yoshikawa et al., 1996, 1998, 2003, 2004a; Abe et. al., 2003; Arissue et al., 2003; Noël et al., 2003). Besides that, current epidemiological and experimental data demonstrate the poor host specificity of *Blastocystis* and proves its transmission from human-to-human, animal-to-human, human-to-animal and animal-to-animal (Parkar et al., 2007). As a result previous nomenclature restricted to species such as *Blastocystis galli* for isolates from chickens, *B. anatis* from domestic ducks, *B. anseri* from domestic geese, etc. is proven inefficient. For these reason, a consensus published on 2007 proposed the use of the term *Blastocystis sp.* followed by a subtype (from 1 to 10) for mammal and avian isolates, including those isolated from humans (Stensvold et al., 2007a). Therefore, as suggested by Yoshikawa et al. (2004b) most of *Blastocystis* isolates from humans and other animals have been accepted as zoonotic parasites.

What we know about the morphology and life cycle of *Blastocystis sp.* mainly originate from studies conducted on *Blastocystis hominis* (Figure 1.), but as noted *B. hominis* is no longer known as a separate species so all the collected data about morphology and life cycle is applicable on *Blastocystis sp.*

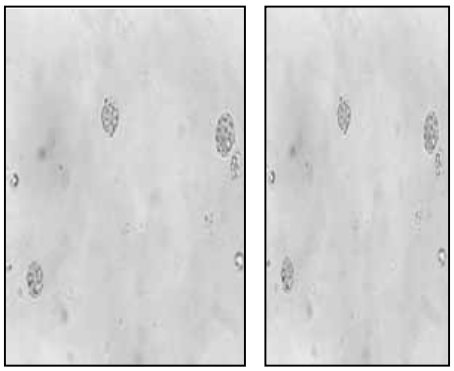

Fig. 1. *B. hominis*, human feces, native preparation, magnification 400x, orig.

Reports of the morphology of *B. hominis* from culture samples commonly have noted three major forms, vacuolar, granular, and ameboid of the organism (Boreham and Stenzel, 1993; Zierdt, 1991; Zierdt et al., 1967). The vacuolar form has been considered to be the typical *Blastocystis* cell form (Zierdt, 1991). The granular form of *B. hominis* has an ultrastructure similar to that of the vacuolar form, apart from having morphologically and cytochemically different central vacuole contents (Dunn et al., 1989; Tan and Zierdt, 1973). Vacuolar and granular forms usually are spherical cells, although irregularly shaped cells may be present in culture samples (Stenzel and Boreham, 1996). Vacuolar forms vary greatly in size, with the average diameter of cells usually being between 4 and 15 ηm (Zierdt, 1991). Granular forms often are slightly larger than the average vacuolar forms, and diameters of 10 to 60 ηm (Zierdt et al., 1967). The vacuolar and granular forms display a thin peripheral band of cytoplasm surrounding a large central vacuole. The organelles usually are found in thickened areas of cytoplasm, often appearing at opposite poles of the cell. These areas of cytoplasm may protrude into the central vacuole (Dunn et al., 1989; Zierdt, 1991) or extend outward to give the cell an irregular outline (Dunn et al., 1989).

Most organelles (Mitochondrion-like organelles, Golgi complex, one or more nuclei) appear to be simple representations of their type (Stenzel and Boreham, 1996). There are several reports about different forms of *Blastocystis*, like multivacuolar and avacuolar forms, ameboid form and a cyst form, but the results are insufficient for a definitive conclusion, although recent studies suggest that the cyst form is likely to be the infective form of *Blastocystis* (Stenzel and Boreham, 1996). This two authors also suggest that binary fission is the only plausible method of reproduction for *Blastocystis* that has been demonstrated. Plasmotomy, endodyogeny, schizogony, and sporulation are not supported by morphological data.

The prevalence of *Blastocystis sp.* infection is high among laboratory rats (60%), pigs (70-95%) and birds (50-100%) (Tan, 2004). The higher prevalence of Blastocystis infection in birds can be correlated to the difference in behavior between birds and mammals, because birds like chickens often eat feed contaminated with feces so it is reasonable to speculate that the chicks are more frequently ingesting the contaminated fecal cysts rather than mammals (Yoshikawa et al., 2004a), and it is known that the primary transmission route is fecal-oral. Also, backyard and free-range chickens easily contaminate drinking water, and by The World Health Organization publication on drinking water quality (2011) Blastocystis sp. is one of the pathogens that must be considered as a waterborne zoonoses.

Animals exhibiting a high prevalence of *Blastocystis sp.* infections may represent an extensive reservoir for infection of humans (Noël et al., 2005). It is proven that experimental animals, such as chickens, can be infected with human-isolate subtypes, thus indicating the zoonotic potential of some human *Blastocystis* isolates (Iguchi et al., 2006). Zoonotic transmission of B. hominis has been speculated since epidemiological studies suggested a conection between close contact with animals and blastocystosis in humans. The mode of transmission is mainly unclear but may be associated with animal contact and with the ingestion of food and water (Lee et al., 2012) contaminated with cysts from reservoir hosts. The studies of Doyle et al. (1990) and Salim et al. (1999) reported that people who work closely with animals do stand at risk of aqcuiring *Blastocystis* infection, and that 44% of examined patients harboring *B. hominis* as the sole enteric pathogen had a history of previous exposure to animals.Thus the high prevalence of *Blastocystis* in chickens represents a significant source for human infection.

The clinical significance of *Blastocystis* in chickens is contradictory because the parasites were isolated from animals showing some clinical symptoms, but also from clinically completely healthy individuals.

In our study we isolated *Blastocystis sp.* from poultry that has been completely healthy, without any symptoms that would indicate intestinal infection, but also from a flock with mild sub-acute to chronic diarrhea. In flocks from which we isolated *Blastocystis* parasites, we did not observe any disturbances in productivity, growth and body weight maintenance. There are several opinions about the mechanism of pathogenesis. Studies have focused on immunological reactions of epithelial cells to proteases secreted by *Blastocystis. Blastocystis* initially down-regulates and then up-regulates production of the inflammatory cytokine IL-8 in epithelial cells (Long et al., 2001), causing gastrointestinal symptoms such as enteritis, colitis and ileitis.

Most clinical methods are based on finding the organism in stool specimens. Variations on this method include concentration, staining, culturing and molecular diagnosis by polymerase chain reaction (PCR) testing (Termmathurapoj et al., 2004). The last one is the most efficient diagnostic technique used, although is more costly, it is known to be more sensitive than the direct smear and xenic culture (Stensvold et al., 2007b). PCR is important not only for diagnosis but also for subtyping, thus some new modified PCR techniques (Santín et al., 2011) can play a great role in explaining the complexity of this genus, its share in human and animal disease, as well as its zoonotic potential. By Roberts et al. (2011) microscopy detects only 48% of the positive samples. If the use of PCR is not feasible for as a diagnostic method, it is recomended that at least two different diagnostic techniques be used for detection of *Blastocystis.* Because of mentioned we need to be cautious when interpreting prevalence reports, especially for studies that rely solely on microscopy.

The requirement for treatment of *Blastocystis* infections remains controversial. In the absence of conclusive evidence of pathogenicity of the organism, treatment with potentially dangereus drugs and the inability to undeniably prove the real causes of symptoms is a significant problem. The treatment should be applied with caution, only after a thorough clinical examination of other possible causes of symptoms. In vitro studies on the effects of drugs, were conducted exclusively in Blastocystis homminis cultures. From 10 antiprotozoal drugs tested, metronidazole proved to be most effective by preventing the production of IL-8. Antibacterial drugs such as ampicillin, penicillin, streptomycin, gentamicin, colistin, vancomycin and antifungal amphotericin B, do not

show any effectiveness against parasites. Considering the present data the most likely route for transmission of *Blastocystis* is the fecal-oral. Thus, control measures must include good hygienic practice, appropriate hygiene in common and sanitary facilities and education to prevent fecal contamination of the enviroment and ingestion of contaminated material (Stenzel and Boreham, 1996).

CONCLUSION

The first stage of are research clearly demonstrates the advantages of keeping poultry on grid floor, and the role of such a system in preventing the development and spread of parasitic diseases.

Based on the results of this research, especially its second phase, as well as the results obtained by other authors we can say that our current knowledge of *Blastocystis* and the subsequent disease that it causes is not enough to determine the importance of parasites in animals. Current literature has to be taken into consideration with a certain caution, because it contains numerous unclear and unconfirmed data. In recent years there has been progress in defining the morphology and life cycle of the parasite, thanks to successful experiments on rats, mice and birds. However, a more detailed knowledge of the biology of organisms is essential for defining effective methods of diagnosis, treatment and control. In future research it is important to determine the infective stage of the parasite and the stages in the life cycle as a suitable target for therapy. It is necessary to accurately determine the function of cell organelles, which can help determine the target point for therapy.

From the clinical aspect, more evidence is needed before we declare *Blastocystis sp.* as the cause of disease in animals. The case studies are of limited value in research, because it is almost impossible to rule out other causes of infections and non-infectious, non-specific symptoms associated with *Blastocystis* infection. Epidemiological findings are significantly limited. It is necessary to determine the true prevalence in different populations of poultry, then determine whether there are different strains that cause symptomatic or asymptomatic disease, and verify the exact mode of transmission. Due to the current lack of incontrovertible data about this organism it is difficult to predict the status of this parasite in the period of 5-10 years, but it is certain that by thorough research may lead to new surprising conclusions.

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CREVNI PARAZITI U INTENZIVNOM ŽIVINARSTVU SA POSEBNIM OSVRTOM NA BLASTOCYSTIS SP.

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Izvod

Prevalenca većine parazitskih bolesti živine signifikantno je redukovana u komercijalnim sistemima uzgoja, kao rezultat poboljšanih uslova držanja, higijene i upravljanja. Međutim, parazitske bolesti su i dalje od velikog značaja kod intenzivnih sistema uzgoja i eksploatacije na dubokoj stelji, odnosno kod slobodnog komercijalnog uzgoja na ispustima, tzv. „free range" a veliki broj parazita može prouzrokovati poremećaje u produktivnosti, kao što su smanjen prirast, loša konverzija hrane, smanjenje nosivosti pa čak i smrtni ishod kod težih infestacija. U praktičnom delu rada izvršen je koprološki pregled fecesa živine različitih proizvodnih kategorija i uzrasta sa farmi na kojima se vrši odgoj i eksploatacija u intenzivnoj formi. Istraživanje je dokazalo prisustvo crevnih parazita Heterakis gallinarum, Ascaridia galli, Eimeria spp. i parazita Blastocystis sp. u fekalnom materijalu živine. Sve što je vezano za ove parazite, osim za Blastocystis sp. nam je manje ili više poznato. Pošto je Blastocystis parazit čija je uloga u živinarstvu tek u nastajanju, diskusija je uglavnom posvećena ovoj zoonotskoj protozoi.

Ključne reči: crevni paraziti, živina, Heterakis gallinarum, Blastocystis.

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