Buxtonellosis and coccidiosis of cattles in Northern Serbia

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Summary

The study presents the results of a two-year research of protozoal infections of cattles from the Northern-Bačka district (Vojvodina, Serbia). The research was conducted on cow-farms and in private cattle-breeding households, with variuos levels of hygenie. The study included 224 specimens (71 calves, 48 hifers and 105 cows). 22,91% of examined heifers and 15,23% of cows were infected with the ciliate *Buxtonella sulcata*. The presence of coccidiosis was detected in 45,07% of the calves, 14,58% of the heifers and 6,67% of the cows. The clinical signs of diarrhoea were established in all of the specimens which had more than 1500 cysts of B.sulcata in 1g of faeces. In the examined calves the number of oocysts was less than 1500 in 1g of faeces, so the coccidiosis did not influence the incidence of the diarrhoea. The results show the first diagnostic record of the ciliate *B.sulcata* of bovines in Serbia, and this finding contributes to the existing knowledge about the etiopathogenesis of protozoal disease of cattle in this area.

Key words: cattle, coccidiosis, *Buxtonella sulcata*, Serbia

Introduction

Coccidiosis of cattle is an acute or chronic disease known in the whole world, and in Serbia it is recorded in isolated cases and in a smaller number of animals (Dimitrijevic and Ilic, 2011). The illness usually manifests itself in a subclinical form, in these occasions it can cause more harm in calves, e.g influencing their growth. In a combination with a stress factor it can become a clinical disease and inflict considerable losses. (Mundt et al.,2007).

The strongest pathogens are: *E.bovis* and *E.zürni*, which in most cases inflict an acute illness. (Dimitrijevic and Ilic, 2011). The species *E.bovis* is localized in the epithelial cells of the small intestine until it reaches the second schizont generation, and its further developement is continued in the large intestine. The species *E.zürni* is localized in the epithelial cells of the cecum, colon and rectum (Mundt et al.2003, Daugschies i Najdrowski, 2005). According to the numbers of infected cattle, for the epizootiology of this disease, the *E.ellipsoidalis* and *E.cylindrica* are also very important (Rehman et al., 2011; Dong et al., 2012). *Eimeria alabamensis* causes illness of calves which are grazing outside, it can occur also in calves kept inside, if they are fed with hay from contaminated pastures (Svensson, 2000)

The ciliate *Buxtonella sulcata* is very similar to a species that occur in pigs and humans (*Balantidium coli*) (Tomczuk et al.,2005). The problem of buxtonellosis in ruminants is not considered a big problem, so there are only a few scientific papers on the pathogenic effects of this ciliate. It is widely accepted that as commensals, they contribute to the process of digestion of plants in the rumen. This explanation is confirmed by constant findings of these ciliates in the digestive tract of ruminants. However, in cases of improper nutrition, the number of ciliates multiply, it starts invading the fithenal parts of the digestive system, causing inflammation and metabolic disorders that can clinically manifest as diarrhea. (Al-Saffar el al., 2010). By analyzing the correlation between the degree of infection and diarrhea, certain authors concluded that B. sulcata cause diarrhea in dairy cows that are infected at a high level (Hong and Youn, 1995; Tomczuk et al., 2005). Recent research suggests that B. sulcata cause diarrhea in calves mainly in the period after 10 days of age, when maternal antibodies cub gets through colostrum, begin to disappear from circulation (Al-Zubaidi and Al-Mayah, 2011). The result of these neonatal diarrheas can be fatal, which is an important factor in intensive calve farming.

The aim of this work is to establish new data about the etipathogenesis of protozooal diseases on the epizootical region of northern Serbia.

Materials and methods

The research was conducted during 2012 and 2013 on cattle farms and in private husbandries in north Backa district (Vojvodina).

Animals. The research was conducted on 224 animals, which were separated in three age categories: 71 calves (younger than 6 months), 48 heifers (age between 6 months and 1 year), and 105 cows. From the aspect of husbandry of the animals, the categories are divided into: animals on rope in a stall, animals partly kept on rope and in surrounded pastures, and animals kept mainly on open pastures. The researched cattle were from different hygienic and husbandry conditions.

Analyzed material. The faeces for the analysis was collected directly from the rectum and was packed in PVC bags, which were marked with appropriate numbers. For every animal the following data was collected: adress and owner of the animal, veterinarian ID number, animal ID number, gender, age, species and colour, husbandry type, feeding method, symptoms of digestive malfunction, dehelmintization and other. Not one of the tested animals was treated with antiparasitic drugs.

Methods of the research. The samples from the suspicious animals were analyzed with qualitative and quantitative methods of coprological diagnostics. From the qualitative methods the methods of concentration of parasitical elements were used (flotation with NaCl and sedimentation). The intensity of the infection was established with the counting of cysts and oocysts in 1g of faeces (Tomczuk et al., 2005).

Statistical data procession. The data was processed in a statistical program: "Graph Pad Prism software". For the determination of statistical difference between the categories of animals which tested positive for parasites the X^2 test was used.

Results

In the conducted research a coprological study of 224 faeces samples was studied, from cattle of different age and husbandry methods. A number of 71 calves, 48 hifers and 105 cows was studied. The prevalence of coccidiosis in calves was 45,07% (71/32). In the heifers the prevalence of coccidiosis was 14.58% (48/7), and of buxtonellosis was 22,91% (48/11). In cows the prevalence of coccidiosis was 6,67% (105/7) and of the ciliate *B.sulcata* was 15,23%(105/16). (Graphicon 1).

From the total number of specimens 20,53% (224/46) was tested positive for coccidiosis, while 12,05% (224/27) was positive for the cysts of *B.sulcata* (Figure 1)

Analyzed by the age categories, the presence of parasites was proved in 45,07% (71/32) in calves, 37,49% (48/18) in heifers and 21,90% in cows (105/23) (Diagram 1).

Based on the results from Table 1, a statistically relevant difference was established in the prevalence ($p<0.005$) between the different categories of animals that tested positive for coccidiosis. In this paper 45,07% (71/32) of the calves was positive for coccidiosis, in

comparison with the cows 7% (105/7) and heifers (48/7). The difference in the prevalence of *Buxtonella sulcata* didn`t prove statistically relevant ($p>0.05$) (Tabel 1).

The intensity between the infection of *B.sulcata* and the occurance of diarrhoea was analyzed and these results were found:

In 50,00% of the animals that tested positive for more than 500 cysts of *B.sulcata* in 1g of faeces, the simptoms of diarrhoea were proven. Diarrhoea was established in 71,43% of tested specimens, in which more than a 1000 of *B.sulcata* cysts were found in 1g of faeces. Also, diarrhoea was found in 100% of the animals in cases where with quantitative methods more than 1500 of these cysts were found in 1g of faeces. (Table 2).

The number of oocysts in the calves didn`t exceed 1500 in 1g of faeces (Table 3).

Discussion

The biggest prevalence with coccidiosis was recorded in the calves, 45,07%. This finding is similar to the data from other literature, that bovine coccidiosis can mostly be found in young and weak animals, and the illness has an acute fluency. In older cattle, there is a formed immunity that protects the animal from this infection (Mundt et al., 2003). The efficiency of this protection can be weakened due to unfavorable circumstances (long transportation, mistakes during transportation, malnutrition, too many animals kept together, the effect of climate factors, unfavorable zoohygienic circumstances). Changes in husbandry can be stressfull for the animals which weakens the immune response, in which case coccidiosis can develop sporadically in older animals (Mundt et al.,2007). This is further proven by our results, which shows that the prevalence of these protosoas in cows is 6,67%.

For the development of clinical signs of coccidiosis the finding of 50000 or more oocysts in 1ml of intestinal matherial or in 1g of liquid faeces. Findings that show a lesser number of oocysts will not demonstrate clinical signs, but it can be a potential source of infection. In the tested calves the diagnosed number of oocysts did not succeed 15000 in 1g of faeces, so the infection with coccidiosis did not influence the apperance of diarrhoea.

The high prevalence of coccidiosis is explained by the fact, that the farmers are not using any anticoccidiosis medicaments which results with the contamination of stalls and pastures and the continued exposure of the animal to the sporulated oocysts of *Eimeria* spp.

The conducted research diagnosed for the first time the presence of the ciliate *B.sulcata* in the epizootical region of Serbia. The results of the research shows that during the 2 year period *B.sulcata* was present in 22,91% heifers and 15,23% of cows.

The large intestine of ruminants is frequently inhabited with the vegetative forms of the phylum Ciliophora, to which *B.sulcata* belongs, the role of this ciliate in the large intestine is unclear. The cysts of this ciliate, which are infective, are forming outside the host. The infection forms by the consuming of food or water contaminated with the faeces of infected animals. In the end part of the small intestine or in the large intestine trophozits are released. The greater the infection is with these pathogens is, the faster the passage is in the intestines, which results in the loss of body mass and diarrhoea. (Hong and Youn, 1995; Wacker et al.,1999).

The high percent of cattle tested positive for this protozao indicates that this is a widely distributed pathogen.Analyzing a wider number of diarrhoea occurance with unknown etiology in diary cows, the results show *B.sulcata* as the main cause. Tomczuk et al. (2005) did a clinical research of cows with diarrhoea, with different level of dehydration, an apetite or thrust disorder, a rumen or rumination disorder, and having problems standing up with some of the animals. The coprological examination proved the presence of cysts of *B.sulcata* in 87,90% of the examinated animals, and the symptoms of diarrhoea were present in 16-17% of the examinated animals.

Huang et al (2012) examined diary cows in Taiwan, and found a prevalence of *B.sulcata* in 61,7% of the tested cows, which grew with the age of the tested animals. Fox and Jacobs (1986) indicate that the density of the population of this ciliate depends on the quantity of carbohydrates in the diet. Al-Zubaidi and Al-Mayah (2011) in neonatal calves in Iraq established the prevalence of *B.sulcata* in 43,20%. In the first few days after birth, the calves receive colostral antibodies from their mother, which can protect them from infection of any kind, including buxtollenosis. As the calf matures, the colostral antibodies dissapear, after 10 days the calf becomes vulnerable to infections, like these ciliates. The speed of the multiplication of *B.sulcata* depends on how low the pH is in the intestines. Two hours after feeding with only milk, the pH in the intestines of calves falls under 6,6, and this helps in the multiplication of these ciliates (Constable, 2009). Buxtonellosis in calves most frequently appears as a mixed infection with cryptosporidiosis. The infection with cryptosporidiosis is the primary infection, and the cows during the birthing period expell oocysts with faeces due to stress (Tzpori, 1988).

Skotarczak and Zielinski (1997) in their research proved the effect of pH in the large intestines on the degree of the infection with *B.coli*. The acidic surround is helping the pathogen to multiply and makes stronger the cytotoxic effect of the parasite. As a result of this the mucous membrane of the large intestine becomes damaged and the inflammation developes further. On the places where the lesions occur, secondary bacteriological infections develop, which lead to even stronger pathological changes. In scientific literature, clinical cases can be found of balantidiosis and the presence of pathological changes in the large intestine in camels, which have similar digestive tracts as cows (Abubaker et al.,2000). The results of experimental infections with this protozooa go in favor of the assumption that similar effects can be expected with buxtonellosis.

Pomajbikova et al.(2012) researched the genetical diversity of intestinal ciliates from the genus *Balantidium* in non-human primates and their host specificity. They analyzed ciliates from

faecal samples from wild primates, primates in captivity and pigs. As the supposed closest relatives to the species *B.coli* the research included *Balantidium entozoon* (which is a parasite of edible frogs) and *Buxtonella sulcata* (which is a parasite of cattle). Based on the polimorphism of SSrDNA sequencies, it has been proven that the species *B.entozoon* filogenetically differs from the species *B.coli*, which has a wide host specifity. That is why for homeotherm organisms a new genus, the *Neobalantidium* was suggested. It was established that the cysts of *Buxtonella*-like ciliates in primates open up a question about the potential spectre of host for the *B.sulcata* and its occurance in humans (Pomajbikova et al.,2013)

The variations of pH can occur because of changes in nutrition, motility problems and dysfunction of the digestive tract. Irregular nutrition that involves an unbalanced meal, that can lead to a multiplication of vegetative forms of *B.sulcata* with a change in pathogenicity. As a result diarrhoea can occur, which aims to balance the content of the intestines and to eliminate parasites (Tomczuk et al.,2005). With the correction of the meal the Ph can be regulated, which lessens the number of parasites in the intestinum. Stronger clinical features indicate a more intensive infection with the protozoo and the presence of secondary bacteriological infection (Al-Saffar et al., 2010)

The finding of ciliate *B.sulcata* in the species that were included in our research can be explained by the method of their nutrition. They were fed once per day, a conplete meal, mixed in a mixer trailer. The meal was based on silage, that could have led to the decline of pH values in the digestive tract of the animals. Added this was luzern and the concentrated part of the meal (corn hominy, wheat bran) and rarely sunflower meal or soybean meal, salt, premix and minazel.

The primary infection with the ciliate *B.sulcata*, which is not a complicated bacteria, can be sanitized with the correction of meals and applying probiotics. Only complicated cases, which include secondary bacteriological infections must be treated with anti-biotics (Tomczuk et al, 2005)

Infections of protozoar ethiology can have unfavorable outcomes followed with high morbidity and mortality of cattle, which result in economic losses. To the favorable outcomes the following factors can contribute: adequate husbandry conditions, nutrition, care and immunoprophilaxis, and, from the veterinary aspect, preventive therapy as a crucial part of cattle farming. Opening the question about the possible spectre of hosts of *B.sulcata* and the possibilities of a human host, the importance of this study grows, from the aspect of human-medicine.

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