9. Dicrocoelium dendriticum in sheep flocks in Novi Sad region: occurrence and estimation of pasture contamination

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Abstract

The lancet fluke, Dicrocoelium dendriticum, causes dicrocoeliosis, an emerging disease often underestimated by researchers and practitioners mainly due to the fact that it is asymptomatic and masked by the pathological effect of multiple parasitic infections in ruminant livestock. Diagnosis is most often based on examination of faecal samples for characteristic eggs, which are very resistant in environment and capable to overwinter. The aim of our investigation was to determine presence of D. dendriticum in sheep grazing in Novi Sad area, and to estimate potential pasture contamination by enumerating eggs after flotation in McMaster slide.

Total of sixty one faecal samples were collected and analysed during January and February 2012 from three flocks: one in city of Novi Sad and two in nearby villages, Bukovac and Kovilj. Sampled ewes grazed during whole season, and they are housed and fed in the winter. About twenty fresh faecal samples from each flock have been collected. The samples were packed separately in plastic cups, transported to the laboratory, and refrigerated until examination. Four gram of each sample was diluted with tap water to ratio 1:10 homogenized and sieved. After mixing, dilution was transferred in plastic tube up to 10 ml, centrifuged, and sediment mixed with saturated zinc-sulphate, spg 1.350, filled to previous level. Eggs were counted in total volume of both chambers (1 ml) of McMaster slide after 10 min. One egg was equivalent to 10 eggs per gram (epg) of faeces (analytical sensitivity = 10 epg). Statistical analysis was performed in Quantitative Parasitology 3.0.

The overall prevalence of D. dendriticum in Novi Sad region was 34.4% (21/61; CI 95%: 22.7%-47.7%), and overall mean intensity was 413.3 epg (95% CI: 323.3 to 515.2). No clinical signs of dicrocoeliosis were observed, except in one sheep in Bukovac which displayed distended abdomen suggesting possible ascites. All investigated sheep from Bukovac were positive (19/19, CI 95%: 82.3%-100%), and mean intensity was 454.7 epg (95% CI: 373.2 to 556.8). In Novi Sad, prevalence was 9.1% (95% CI: 1.1%-29.2%), with mean intensity 20.0 epg (95% CI: 10.0 to 20.0). In investigated flock from Kovilj there were no positive sheep at time of research.

Results of our study show that dicrocoeliosis is present in sheep flocks from Novi Sad region. At this moment, according to the results of egg enumeration, the highest pasture contamination possibility was estimated in Bukovac, while very low and

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none were found for Novi Sad and Koviž, respectively. These findings impose the need for further investigation of dicrocoeliosis for regular update of parasitological status of sheep flocks and for successful control. Treatment with appropriate anthelmintic drugs is necessary to avoid high pasture contamination in the beginning of grazing season.

Key words: Dicrocoelium dendriticum, sheep, pasture contamination, Novi Sad region

Introduction

The lancet fluke, Dicrocoelium dendriticum, causes dicrocoeliosis, an emerging disease often underestimated by researchers and practitioners mainly due to fact that it is asymptomatic and masked by the pathological effect of multiple parasitic infections in ruminant livestock (Otranto and Traversa, 2003). D. dendriticum causes no clinical illness in lambs or yearling sheep, but these hepatic trematodes are long-lived and the pathological changes in the liver increase in severity and extent with the duration of the infection (Bowman, 2009). Therefore, dicrocoeliosis is manifested in older (adult) sheep, with the economic and health importance due to direct losses caused by condemnation of infected liveweight and deaths of sheep at rare occasions (Otranto and Traversa, 2002) and indirect costs derived from hepatitis alterations caused by the parasite, such as a decrease in animal weight, delay in growth, reduced milk yield and costs due to anthelmintic treatment (Manga-Gonzáles et al., 2007). This parasitosis belongs to the six principal and economically most important pasture helminthoses of sheep (Duchâteck et al., 2003) and its complex life cycle with two intermediate hosts (terrestrial snail-more than 100 species; ant-at least 21 species mainly from the genus Formica (Manga-Gonzáles and González-Lanza, 2005)) makes it very widespread. Reports of dicrocoeliosis are increasing mainly due to expansion of dry habitats and the increase of anthelmintic resistance (Otranto and Traversa, 2003).

Diagnosis of dicrocoeliosis is most often based on examination of faecal samples for characteristic eggs and quantification is the most often performed after flotation in McMaster slide (Rehbein et al., 1999). D. dendriticum eggs are very resistant in environment and capable to overwinter and remain infectious up to 20 months on pasture (Otranto and Traversa, 2003), so, in the spring, viable eggs enable infection of first intermediate host which leads to increased risk for reinfection of grazing animals as cycle continues.

The aim of our investigation was to determine presence of D. dendriticum in sheep grazing in Novi Sad area, and to estimate potential pasture contamination by enumerating eggs after flotation in McMaster slide.

Materials and methods

Total of sixty one faecal samples were collected and analysed during January and February 2012, from three flocks in Novi Sad region, Vojvodina, Serbia: one in city of Novi Sad and two in nearby villages, Bukovac and Koviž. Samples were taken during whole season, and they are housed and fed in the winter. About twenty fresh faecal samples from each flock have been collected from the ground within two minutes after defecation, as described by Abbott et al. (2009). Collection of samples was obtained in the morning (range from 08:00 - 11:00h). The samples were packed separately in plastic cups, transported to the laboratory immediately, and refrigerated until coprological examination.

Four gram of each sample was diluted with tap water to ratio 1:10, homogenized and sieved through tea strainer to remove heavy debris. After mixing, dilution was transferred in plastic tube up to 10 ml, centrifuged 2 min at 1500 rpm, supernatant discarded and then flotation solution (saturated zinc-sulphate, specific gravity 1.35) was filled in tubes to the previous level, and thoroughly mixed with remaining sediment. Eggs were counted in total volume of both chambers (1 ml) of McMaster slide after 10 min. One egg was equivalent to 10 eggs per gram (epg) of faeces (analytical sensitivity=10 epg). Statistical analysis was performed in Quantitative Parasitology 3.0.

Results and discussion

The overall prevalence of D. dendriticum in Novi Sad region was 34.4% (21/61; CI 95%; 22.7%-47.7%), and overall mean intensity was 413.3 epg (95% CI: 323.3 to 515.2). No clinical signs of dicrocoeliosis were observed, except in one sheep in Bukovac which displayed distended abdomen suggesting possible ascites. All investigated sheep from Bukovac were positive (19/19; CI 95%; 82.3%-100%), and mean intensity was 454.7 epg (95% CI: 373.2 to 556.8). In Novi Sad, prevalence was 9.1% (2/22, 95% CI: 1.1%-29.2%), with mean intensity 20.0 epg (95% CI: 10.0 to 20.0). In investigated flock from Koviž there were no positive sheep at time of research. Overall minimum and maximum egg counts were 10 and 980, respectively. Range of egg elimination in Bukovac was 90-980 epg, and in Novi Sad 10-30 epg.

Prevalence of dicrocoeliosis in several coprological investigations in Europe is different: from very low values of 7% in Sardinia (Italy) (Sánchez-Andrade et al, 2003), moderate (25% of sheep infected with D. dendriticum) in North-Western Spain (NW Spain) (García-Pérez et al., 2002), to high values found in Bosnia (74.1% of investigated sheep in slaughtered sheep (Zuko and Hodžić, 2011), 83.25% in Serbia (in goats at Belgrade area) (Pavlović et al., 2011). Manga-Gonzáles et al. (2007), in other study in NW Spain, determined that 100% of investigated sheep excreted eggs at least once during research. In our study, prevalences in two positive flocks from Bukovac and Novi Sad were 100% and 9.1%, respectively. These differences could be explained with several facts. Firstly, there is lack of sensitivity in in vivo diagnosis based on coprological techniques (Broglia et al., 2009) and negative findings are not always evidence of parasitological absence. Coprological method was proved to detect the presence of dicrocoeliosis in one out of three infected sheep (Duchâteck et al., 2003). Also, applied technique may have influence on number of quantified eggs in faeces. Thus, flotation with high density solutions with specific gravity 1.30-1.45 is more efficient than sedimentation (Otranto and Traversa, 2003). When considering flotation solutions, better results were obtained using HgI2/KI (spg 1.440) than with ZnSO4 (spg 1.350), but former solution is toxic to man and environment because of mercury, and restricted use is justified (Rehbein et al, 1999). Further, according to some authors, the number of eliminated eggs in experimentally infected lambs was higher in the afternoon than in the morning. (Campo et al., 2000, Şenlik et al, 2006).
Long pre-patent period (in experimentally infected lambs was 49-79 days (Campos et al., 2000)) also leads to false negative results. If sheep is infected with less than 100 flukes eggs will usually not be detected (Broglia et al., 2009).

There is difference in egg elimination by sheep host during the year. In order to determine the period of the year when pasture contamination with viable D. dentrificum eggs was high, authors in NW Spain followed egg elimination kinetic. They have determined that the highest percentage of animals eliminated eggs in February (86.66%) with the highest mean epg (around 375 epg) and the lowest in July (around 130 epg). Mean number of excreted eggs increased in autumn and reached maximum in the end of the winter (Manga-Gonzáles et al., 2010). In the same region, García-Pérez et al. have found much lower egg elimination with mean egg output of 43 epg (egg output between 0 and 1650 epg) (García-Pérez et al., 2002). Results of egg quantification obtained in Bukovac and Novi Sad, are close to those gained by Manga-Gonzáles et al. in February i.e. those measured by García-Pérez et al., respectively.

Positive correlation between egg count and total fluke count was observed by Campo et al. (2000) and Şenlik et al. (2006). D. dentrificum is long lived parasite and pathological changes (chronic irritation) increase in severity over time if left untreated. Long term infections cause progressive hepatic cirrhosis, shorten reproductive life of sheep and decrease wool production and lactation. Still, there is no clinical significance if there are less than 1000 flukes in liver of lambs (Manga-Gonzáles and Gonzáles-Lanza, 2005). It has been reported that burdens of up to 4000 parasites do not cause significant blood or plasma loss in sheep (Otranto and Traversa, 2002). Nevertheless, there are recent reports of deaths in sheep due to dicrocoeliosis in Vojvodina (Cegledi, 2012) personal communication).

So, if we consider facts that one adult sheep excretes 1-3 kg faeces/day (Stojic, 1999) and that, theoretically, one egg can produce up to 400 000 of adult parasites (Ducháček et al., 2003), it is clear that high epg values can lead to intensive pasture contamination. In some mountain areas, during grazing season animals are spread in broad areas so that faecal excretion is dispersed and pasture contamination is kept at low levels. In winter, the system gets intensified during lambing and milking, and animals graze near the farm in more restricted area with higher level of parasite contamination (García-Pérez et al., 2002).

Grazing area and feeding practices of farmers in Vojvodina vary between different farms and localities. We observed that in Bukovac, some farmers feed sheep with grains on pasture near barn, so that in the period of the highest egg elimination intensive contamination of farm surroundings may occur. According to Vujic (1972), sheep are main contaminators of pastures with D. dentrificum eggs in Vojvodina. Since there are smaller and larger grazing areas, depending on locality of the farm/flock, with different number of grazing animals and stocking rate, this will lead to variable pasture contamination. Differences of prevalence of D. dentrificum in three localities from our study may be to different type of pastures. Pastures in Novi Sad and Kovič (72-80 m and 89 m above mean sea level (AMSL), respectively) may be referred as lowland, and pasture in Bukovac as upland pasture (224 m AMSL).

Periods when temperatures are low favor D. dentrificum viability in the field as they are more resistant to low temperatures than to high ones (they can stand temperatures as low as -20°C to -50°C) (Manga-Gonzáles et al., 2007). The high contamination of pastures which occurs during autumn and winter facilitates viable egg ingestion by snails, which start to be active and are very abundant in the spring. Ants could eat slime balls containing cercariae and if metacercariae are developed they can be eaten until the end of ants' active period in November. As a consequence of this, egg excretion reaches its highest values in January-February, that is, about two months after the last ingestion of the infected ants before hibernation starts (Manga-Gonzáles et al., 2010).

Likewise, studies have shown that egg mortality in faeces exposed in the environment during hot months of July and August is nearly 100% (Manga-Gonzáles et al., 2007). So, if pasture contamination of viable eggs is reduced to a minimum in strategic time, this will reduce the probability of mollusc intermediate hosts to be infected in the spring, just when they start to be active and are very abundant.

Conclusion
Results of our study show that dicrocoeliosis is present in sheeps flocks from Novi Sad region. At this moment, according to the results of egg enumeration, the highest pasture contamination possibility was estimated in Bukovac, while very low and none were found for Novi Sad and Kovič, respectively. These findings impose the need for further investigation of dicrocoeliosis for regular update of parasitological status of sheep flocks and for successful control. Treatment with appropriate antihelmintic drugs is necessary to avoid high pasture contamination in the beginning of grazing season.

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**Dicrocoelium dendriticum** у стадима оваца из околнине Новог Сада: присуство паразита и процена контаминације пашњака

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Кратки садржај

Мали метић, *Dicrocoelium dendriticum*, узрочник је дикроцелиозе, све учесније болнести често поштовањем од стране истраживача и ветеринара практичара због чињенице да код преживљава протиче асимптотски, маскирајући патолошким ефектима мешаних паразитских инфекција. Дијагноза болести најчешће се поставља на основу прегледа фекеса на присуство карактеристичних јаја која су веома оптимна на услове спољашњег средине и способна да презиме. Циљ нашег истраживања је био да испитамо присуство *D.dendriticum* код оваца које се налазе у околнини Новог Сада и да проценимо могућу контаминацију пашњака бројањем јаја након флоратије у *McMaster* коморишама.

У току јануара и фебруара 2012. године сакупљен је укупно 61 узорак фекеса из три стада: једног у Новом Саду и два из оближњих села, Буковца и Ковиља. Ове од којих су сакупљен узорци на процењено током четири сезона, а у току зиме крајење у затвореном систему држања. Од сваког стада узето је око двадесет узорак свежих фекеса. Узорци су упаковани у пластичне чаше у кома су транспортовани до лабораторије где су хране у фрижидер до испитивања. Четири грама сваког узорка помешано је са водом до разређења 1:10, хомотенирано и процењено. После мешања, у пластичне спремнице је пренето по 10 мл од сваког разређеног узорка, центрифугирани, након чега је седимент јолат засипан раствор цинка сулфата, упркос 1,350, до 10 мл. Након 10 минута, јаја су бројана на нивоу читаве запремине обе коморишта (1 мл) *McMaster* спрема. Једно јаје је еквивалент за 10 јаја по граму (епг) фекеса (апаратичка осетљивост=10 епг). Статистичка анализа урађена је у програму *Quantitative Parasitology* 3.0.

Укупна превозена *D. dendriticum*-а за регион Новог Сада износила је 34.4% (21/61; CI 95%: 22.7%-47.7%), док је укупна просечна инфестација износила 413.3 epg (95% CI: 323.3 do 515.2). Клинички значани дикроцелиозе нису примећени ни једном случају, осим код једне овце у Буковцу са изразито увећавањем амплета који је указивао на могући аспиртес. Све испитане овце од Буковца показале су се позитивним (19/19, CI 95%: 82.3%-100%), са просечним инфестацијом од 454.7 epg (95% CI: 373.2 do 556.8), у Новом Саду превозена је износила 9.1% (95% CI: 1.1%-29.2%), а просечна инфестација 20.0 epg (95% CI: 10.0 do 20.0). У испитованом стаду из Ковиља није било позитивних овака у тренутку истраживања. Резултати нашег истраживања показују да је дикроцелиоза присутна у стадима оваца из околнине Новог Сада. У овом тренутку, према резултатима бројања јаја, могућа контаминација пашњака процењена је као најинтензивнија у Буковцу, док је у Новом Саду, односно Ковиља, процењена као веома слаба или никаква. Овајх налаз намешта потребу за даљим испитивањем дикроцелиозе у циљу редовног аукурирања паразитолошког статуса стада овака и њене успешне контроле. Третман одговарајућим антихелимитицима неопходан је како би се избегла висока контаминација пашњака на почетку пашне сезоне.

**Кључне речи:** Dicrocoelium dendriticum, овце, контаминација пашњака, регион Новог Сада