

# Protozan Infection in Flocks of Small Ruminants in Belgrade Area During 2020

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## Abstract

During our research we examined flocks of small ruminant originated from 23 villages from city districts Mladenovac, Lazarevac, Obrenovac, Grocka, Zemun, Surčin, Palilula, Vozdovac and Zvezdara. In more than 80 percent of the herds, sheep and goats were breed together at same pasture. Using standard coprological methods we examined 273 faecal samples from 41 herds. Determination of parasites we performed by morphological characteristics. Molecular detection of *Cryptosporidium* sp. and *Giardia* sp. we not performed. Coccidiosis was found at 27 herds. We usally ocured mixed infection with 2-3 coccidia species. At sheep most abundant coccidia were *E. faurei*, followed by *E. ovinoidalis*, *E. pallida* and *E. ahsata*. At goats most abundant species were *E. arlongy*, folwed by *E. nina-kohl-yakimovae*, *E. hirci* and *E. caprina*. Clinical signs of disease were present only at young animals but oocysts were found at both, adult ant young animals. *Cryptosporidium spp.* was found at 19 herds Clinical signs of cryptosporidiosis were established only at young animals. They had moderate morbidity and mortality rate. Infection with *Giardia duodenalis* was found only at 2 herds. *Giardia*-infected animals generally had no clinical symptoms.

**Keywords:** Belgrade, cryptosporidium, Eimeria, giardia, small ruminants.

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## 1. Introduction

The suburban municipalities of Belgrade are inhabited by numerous villages where agriculture and animal husbandry are the main economic branches. Breeding of sheep and goats were increased during last decade on Belgrade area. Today, small flocks of sheep and goats play an important role in providing animal protein for diet, especially for those people who live in village in the surrounding environment of Belgrade. Both, sheep and goats are milked and they produce the bulk milk supply, together with a large proportion of the meat that is consumed [1,2,3,4].

During earlier research on their parasitic fauna, the emphasis was on gastrointestinal and lung parasites and ticks. Studies of the prevalence of protozoan infections have not been fully addressed. Only one be performed in period 2011-2012 [5] and after that this research stopped, and therefore, the aim of our study performed during 2019 was to determine the prevalence and types of protozoan infections of small ruminants in Belgrade area.

## 2. Materials and methods

Geographical and climate data about examined area was next: Belgrade is situated in South-Eastern Europe, on the Balkan Peninsula. It lies at the point where the river Sava merges into the Danube, on the slope between two alluvial planes.

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The river waters surround it from three sides, and that is why since ancient times it has been the guardian of river passages. Belgrade lies 116.75 meters above sea level and is located at confluence of the Danube and Sava rivers. The city has an urban area of 360 square kilometres, while together with its metropolitan area it covers 3,223 km<sup>2</sup>. On the right bank of the Sava (examined area), central Belgrade has a hilly terrain, while the highest point of Belgrade proper is Torlak hill at 303 m. The mountains of Avala (511 m) and Kosmaj (628 m) lie south of the city [6]. Across the Sava and Danube, the land is mostly flat, consisting of alluvial plains and loessal plateaus. The surroundings of Belgrade have a large area of pastures where sheep and goats can graze freely. Grasslands are mostly dominated by unsown communities of wild plants and are most often natural or semi-natural habitats. Although their plant communities are natural, their maintenance depends on anthropogenic activities such as grazing and mowing. The diet of small ruminant on the pastures dominates mostly in the plains and in the hilly regions around Belgrade.

The study of endoparasites infection we were carried out in 41 herds from 23 vilages from city districts Mladenovac, Lazarevac, Obrenovac, Grocka, Zemun, Surčin, Palilula, Vozdovac and Zvezdara. Examination we performet in next villages: Mladenovac, Vlaška, Mala Krsna, Velika Krsna, Međulužje, Senjak, Velika Ivanča, Orašac, Mala Vrbica, Rajkovac, Dubona, Šepšin, Kovačevac, Velike Granice, Granice, Koraćica, Jagnjilo, Markovac, Lazarevac, Arapovac, Junkovac, Leskovac and Sokolovo. The largest number of herds (29) numbered from 10 to 20 animals and only 12 herds had more than 20 animals. A total of 25 herds of sheep, 14 herds of goats and 2 herds of both sheep and goats were examined. The research was conducted throughout the year from January to December 2019.

We examined 273 faecal samples with faecal concentration techniques, especially zinc sulphate flotation, and with sedimentation technique [7]. Direct smear or wet mount examination for oocyst and trophozoites can also be performed. However, because of the cyclical nature of cyst excretion, several samples need to be examined to detect the organism.

The diagnosis of parasites is commonly established by microscopic identification of

oocyst, cysts or less commonly trophozoites in faecal wet smear stained with iodine [7]. The numbers of oocysts per gram (OPG) of faeces were determined by the McMaster technique and identification of oocysts was made on sporulation. Determination of subspecies of cryptosporidia and giardia we not performed.

Examinations we performed with AxioLab A1 microscope with the AxioCam 105 Color microscope camera and Zen Lite software, manufactured by Carl Zeiss.

### 3. Results and discussion

During our examination, coccidiosis was found at 27 flocks. Coccidia were found in 16 flocks of sheep, 9 flocks of goats and in both flocks where goats and sheep were kept together. Usually, mixed infection with 2-3 coccidia species occurred, and less often with more species.

At sheep most abundant species were *E. faurei* found at 56%, followed by *E. ovinoidalis* at 43%, *E. pallida* and *E. ahsata* at 37%. Other eimeria species (*E. ovina*, *E. ovinoidalis* and *E. unctata*) were occurred in lest of 5 %. Multiple-species infections were detected in 85.44% of positive sheep which carried two to five species; 77.12% of positive animals had two to three species and infections with 4 and 5 species were les then 2%. The prevalence of *Eimeria* oocysts in male was higher (57.21%) than that in female (51.11%). Also, the OPG number was higher in male (157.32 ± 21.04) when compared to female (151.97 ± 29.79). The prevalence of *Eimeria* oocysts in <6-months old age categories were 61.56 %, and rate of infection was 341.11±53.44 (0-1785), in 6-12-months old prevalence were 52.35% and rate of infection was 104.42±18.87 (0-1123) and in >12-months old prevalence was 45.21% and rate of infection was 61.12±13.49 (0-519). The highest prevalence was recorded in the youngest age category (<6-months old) and the lowest one was observed in the oldest age category (>12-months old).

Usually, poor management is the reason why coccidia numbers increase excessively; thus, coccidiosis may be considered a man-made disease [8,9,10]. This also suggests that coccidiosis can be adequately managed [11,12]. At sick animals, dominant symptoms of coccidiosis range from loss of appetite and slight, short-lived diarrhoea to severe cases involving

great amounts of dark and bloody diarrhoea and, in some cases, death [13]. At numerous cases, diarrhoea begins pasty, then becomes watery and those lambs may dehydrate rapidly [14].

Compared to previous research, the number of *Eimeria* species has decreased, but not their prevalence. This was influenced by microclimatic conditions, changes in the composition of the herd and the application of modern zootechnical measures. In the period 2011-2012, sheep were procured from different parts of Serbia without adequate parasitological control and this resulted in a large number of species of coccidia that originated from different regions from which the sheep originated. During that examination we established the presence of *E. pallida*, *E. parva*, *E. unctata* and *E. rachmatullinae*, *Eimeria ahsata*, *E. ammonis*, *E. arkhari*, *E. crandallis*, *E. dalli*, *E. danielle*, *E. faurei*, *E. gilruthi*, *E. gonzalezi*, *E. granulosa*, *E. intricata*, *E. marsica*, *E. ovina*, *E. ovinoidealalis* [5]. Ten years later, when stable herds were formed, which are permanently present here, and with the application of adequate zootechnical and preventive measures, this number of species decreased significantly, as did the economic consequences of coccidiosis.

At goats most abundant species were *E. arlongy* 59%, followed by *E. nina-kohl-yakimovae* at 53%, *E. hirci* at 49% and *E. caprina* at 25%. Other coccidia species like *E. alijeve* were infrequently found. The OPG number was higher in male ( $166.12 \pm 24.14$ ) when compared to ( $152.17 \pm 21.39$ ) female, for both kids and adult. The prevalence of *Eimeria* oocysts in goats <6-months old age categories were 64.46 %, and rate of infection was  $321.21 \pm 55.44$  (0-1627), in 6-12-months old prevalence were 60.39 % and rate of infection was  $114.92 \pm 17.27$  (0-1213), and in >12-months old prevalence was 43.81% and rate of infection was  $69.72 \pm 15.49$  (0-559). The highest prevalence was recorded in the youngest age category and the lowest one was observed in the oldest age category. Multiple-species infections were detected in 88.24% of positive goats which carried two to four species; 53.42% of positive goats had two to three species. Infections with 4 species were less than 3%.

Oocysts were found at adult and young animals, but clinical sign of disease were present only at young animals. Signs often show 2-3 weeks after the animals are weaned, because the lactic acid

produced by the digestion of milk helps to inhibit coccidia in the nursing animals.

With goat herds where the parasitological picture has greatly improved compared to previous research. During first examination at goats were established *Eimeria absheronae*, *E. africensis*, *E. alijeve*, *E. arloingi*, *E. babaevi*, *E. caprovina*, *E. christenseni*, *E. hirci*, *E. jolchijevi*, *E. kocharii*, and *E. nina-kohl-yakimovae* and ten years after *E. alijeve*, *E. arlongy*, *E. nina-kohl-yakimovae*, *E. hirci*, *E. caprina* and *E. christenseni*.

*Cryptosporidium spp.* was found at 19 herds – 11 herds of sheep 6 herds of goats and in both herds where goats and sheep were kept together

Cryptosporidiosis is parasitic disease with clinical signs at kids or lambs between five and twenty-one days old are the most susceptible [15,16]. The extent to which a kid is infected seems to be dependent on its age and immune status. Younger animals are much more susceptible to infection than adults. In studies done with lambs, five-day-old lambs had diarrhoea for 9-10 days and suffered from a high rate of mortality. Sixty-day-old lambs showed no symptoms when they were infected and adult sheep completely resisted infection. There is an indication that adults develop immunity to *Cryptosporidium*, yet this immunity does not seem to be passed to their offspring [17,15,18].

Once kids become infected, they pass oocysts in their faeces in about five days. The primary mode of transmission is by faecal-oral spread. Animals eating faecal-contaminated material (food, water or bedding) can become infected. If the next kid coming into the same housing area licks on the contaminated walls, it can become infected. Oocysts are thought to survive for long periods of time outside the animal.

At young animals, cryptosporidiosis had high morbidity and mortality rate. Symptoms of acute cryptosporidiosis include lack of appetite, and weight loss. Consequence are significant increase of its accrescent, weakens and less develop [13,19].

Clinical signs of cryptosporidiosis were established only at young animals. Symptoms of acute cryptosporidiosis include lack of appetite, weight loss, and diarrhoea which is usually yellow to yellowish-brown in colour and of a creamy texture [20]. The rapid loss of nutrients and fluids during diarrhoea results in severe dehydration [21]. Some animals do not develop into chronic

cases and become carriers [13]. After infection, animals resist the organism, develop a mild infection that is self-limiting, or soon sicken and die [16,22].

Infection with *Giardia duodenalis* was found only at 2 flocks of sheep and goats breed together. *Giardia*-infected animals generally had no clinical symptoms. The appearance of giardiasis in small ruminant herds has been observed only in the last few years. In small ruminants, giardiasis more surveys from sheep than goat populations and therefore fewer publications on giardia in goats [23,24,25,26,27].

First cusses of infection of sheep we established during 2019. Infection we found in pre-weaned lambs ( $\leq 3$  months) having a much higher compared to those that were over 3 months. In infected lambs we found clinical signs like foul-smelling diarrhoea which is lightly coloured, greasy and mixed with mucous and reduced weight gain [2]. First cases of giardiasis at goats we established during 2018 [28].

At goat giardiasis usually result with diarrhoea, especially in young animals, which in turn adversely affect production resulting in economic loss. At the same time, diseased of small ruminants pose an epidemiological danger because they excrete infectious forms of *Giardia* in their faeces [17,26,29]. In the all cases in therapy were used fenbendazole an effective molecule, at least in terms of complete clinical recovery.

They confirming observations of other authors, who successfully treated sheep and goats infected with *G.duodenalis* using similar therapy protocols.

#### 4. Conclusions

Enteral protozoan infection was of great importance to health status of small ruminants and its performances. Infection usually had moderate morbidity and low mortality rate. Consequence is significant increase of lambs and kid accrescent, its weakens and less growth. The best preventive measures a sheep and goat producer can take is to use a feed with a coccidiostat added. In the all cases of giardiasis in therapy were used fenbendazole an effective molecule, at least in terms of complete clinical recovery. With careful management and sound preventive measures, the losses associated with this disease can be reduced to minimal level.

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