VOL IV

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

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APRESENTAÇÃO

É com grande satisfação que apresentamos o volume IV da coletânea **Estudos em Ciências Agrárias e Ambientais**, resultado do esforço colaborativo de pesquisadores de diferentes regiões e instituições, que compartilham aqui reflexões, dados e contribuições relevantes para o avanço do conhecimento técnico-científico em suas áreas de atuação.

Este volume reúne 13 trabalhos organizados em cinco eixos temáticos que refletem a diversidade e complexidade do campo agrário e ambiental contemporâneo: Sistemas de Produção Aquático e Animal; Sustentabilidade Ambiental e Conservação de Recursos Naturais; Sistemas de Produção Vegetal e Agricultura de Precisão e Educação e Inovação no Meio Agrário.

Os temas abordados vão desde o manejo sustentável de recursos naturais, passando por inovações tecnológicas na agricultura e aquicultura, até discussões sobre formação profissional e segurança sanitária nas cadeias produtivas. Essa pluralidade é o reflexo da crescente interdisciplinaridade que caracteriza os estudos agrários e ambientais hoje – exigindo diálogos entre a ciência, a tecnologia, a educação, a economia e a sociedade.

Além da qualidade dos estudos apresentados, destacamos o compromisso dos autores com a pesquisa aplicada, a sustentabilidade e a busca por soluções adaptadas às realidades locais, muitas vezes desafiadoras. A presença de autores da América Latina e Europa também fortalece o caráter internacional da obra, fomentando o intercâmbio de experiências e metodologias.

Agradecemos a todos os autores pela confiança em compartilhar seus trabalhos conosco. Que esta publicação possa inspirar novas pesquisas, colaborações e, acima de tudo, práticas que contribuam com a construção de sistemas agrários e ambientais mais resilientes, justos e inovadores.

Desejamos a todos uma excelente leitura!

Eduardo Eugênio Spers

SUMÁRIO

CAPÍTULO 4

CONTROL PROGRAM OF SHEEP COCCIDIOSIS IN THE PRODUCTION CHAIN FROM THE BREEDER TO THE CONSUMER

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ABSTRACT: Sheep farming holds significant economic and cultural value in Serbia, with increasing attention given to improving health and production outcomes. Among the challenges faced in sheep husbandry, parasitic diseases - especially protozoan infections like coccidiosis caused by *Eimeria* and *Cryptosporidium* species -are prevalent and economically detrimental, particularly in young lambs. This study presents a comprehensive, multi-year control program for ovine coccidiosis, implemented across nine farms from 2014 to 2018. The program combined systematic parasitological monitoring, targeted therapeutic interventions (notably the use of Toltrazuril), and the application of biosecurity, zootechnical, and prophylactic measures in both pens and pastures. Initial diagnostics showed a high prevalence of subclinical and clinical infections, with lambs being the most affected group. Through successive parasitological examinations and treatments, a significant reduction in oocyst shedding and clinical cases was achieved. By fostering natural immunity through controlled subclinical exposure and timing of therapeutic intervention, the program effectively transformed coccidiosis from an acute production-limiting disease to a managed endemic condition. The study highlights the importance of integrated, farm-specific health strategies and underscores the role of veterinarians in implementing sustainable parasite control measures in sheep flocks in Serbia.

KEYWORDS: coccidiosis control; sheep health; Eimeria infection; Toltrazuril treatment; parasitic disease management.

1. INTRODUCTION

Sheep breeding is an important branch of livestock production in Serbia. Despite the fact that the number of sheep and goats in the social and individual sector of production varies from time to time, this branch of the economy and its improvement is being paid more and more attention. The reason for this lies not only in tradition, but also in the knowledge that the breeding of small ruminants represents a significant economic item, both due to the production of wool and milk, as well as lamb meat, a highly sought after item on the world market.

The improvement of this production is related to solving a number of different problems, which aim primarily to increase the economy while preserving the health and well-being of the animals. Within the scope of these tasks, primary attention must certainly be paid to health care, including the suppression and control of parasitic infections, among which protozoa occupy a significant place, and some are also zoonoses.

The stable method of keeping small ruminants with a large number of animals in a relatively small space, with a uniform microclimate and a deep mat favors contamination with protozoan pathogens and infections, especially coccidia, especially for younger categories of animals. Based on research in the world and in our country, diseases of parasitic etiology dominate in sheep both in terms of prevalence and incidence, accompanied by significant morbidity and low to moderate mortality.

The damage that occurs in this production is a consequence of the indirect negative pathogenic effects of the parasite on the host organism. In favorable conditions for the development and survival of paraparasitic stages in the external environment, the conditions for infections of greater intensity, often with a greater number of parasite species, with different localization in the host organism are obtained. The consequence of this is the development of a clinically manifested disease, with the death of a large number of individuals, most often among the younger categories.

The fact is, however, that in most cases, parasitic infections occur subclinically, that is, “imperceptibly” to the eye of the herdsman. Negative economic effects are also present in these situations and are manifested by a decrease in animal production, i.e. a decrease in the production of wool and milk, poorer grooming of young animals, a decrease in general body resistance, i.e. increased susceptibility to agents of other etiologies.

2. COCCIDIOSIS OF SHEEP

When we say coccidiosis, we usually mean the disease caused by protozoa from the family phylum *Apicomplexa* - genus *Eimeria* and genus *Cryptosporidium*.

Coccidia are intracellular parasites strictly specific for the host species and for the segment of the digestive tract in which they parasitize. Unsporulated oocysts (and therefore non-infectious) are excreted in the faeces of infected individuals. Apart from the *Eimeridae* family, the coccidia genus includes far more dangerous zoonotic species - *Cryptosporidium* sp., *Toxoplasma* sp. and *Sarcocysta* sp. but they are present in a small percentage, except for the genus *Cryptosporidium*, in sheep flocks in Serbia. As coccidia from the genera *Eimeria* and *Cryptosporidium* are the primary and most important causes of illness in lambs, our goal was aimed at their control.

The genus Eimerida are intracellular parasites strictly specific for the host species and for the segment of the digestive tract in which they parasitize. Unsporulated oocysts (and therefore non-infectious) are excreted in the faeces of infected individuals. The life cycle of coccidia is direct and very short. It lasts less than 7 days. Oocysts expelled in faeces sporulate in the external environment in 1-2 days (this is called sporogony and during it sporocysts are created, the number of which is characteristic for each genus).

When susceptible individuals ingest infectious oocysts, sporozoites are released in the digestive tract. They enter intestinal epithelial cells, round up, grow and become first-generation schizonts. This is how asexual reproduction begins - schizogony, which takes place through several generations of schizonts that produce a large number of primary merozoites, which leave the host cell, enter a new epithelial cell, round up, grow and produce secondary schizonts. They further produce large numbers of second-generation merozoites, which exit the host cell. Some enter new intestinal epithelial cells, round up and become third-generation schizonts, which produce third-generation merozoites. From the largest number of merozoites of the II generation, macro- and microgametocytes are formed, which initiates sexual reproduction - gametogonia. The maturation of macro- and micro gametocytes gives rise to micro and macrogametes. Microgametes fertilize macrogametes and a zygote is formed. The zygotes mature and form young oocysts, which are excreted in the feces. The oocysts then sporulate and are ready for a new round of infection.

Coccidiosis finds very favorable conditions for its development in the modern livestock production system, where a large number of individuals are kept in a small space.

Only depending on the fulfillment of zoohygienic and zootechnical conditions and preventive measures used in certain types of production, coccidiosis will pass in a latent form or will be in a clinically manifest form followed by a large death.

Sheep coccidiosis occurs in an acute and chronic course, with high morbidity, a rarer clinical manifestation occurring in lambs. Adult animals do not show a clinical picture of coloring, but they are transmitters of this disease to younger categories.

The causative agents of sheep infections are: *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. ninakohlyakimovae, E. ovina*, *E. ovinoidalis, E. pallida*, *E. parva*, *E. punctata* and *E. rachmatullina.*

Unsporulated oocysts are excreted in the feces of infected individuals, which sporulate in the external environment and become infectious. Goats are infected with oocysts through contaminated food and water.

The site of parasitism is the small intestine. The clinical picture is present only in younger animals when there is greenish or yellow watery diarrhea with an unpleasant smell, and sometimes blood is also present. Abdominal pain, sometimes anemia, loss of appetite, dehydration, tenesmus, weakness and weight loss, depression and inactivity in the form of diarrhea up to exhaustion occur (occurs only in severe infection). The disease is characterized by a moderate number of infected animals and low mortality. The pathoanatomical finding consists of edema of the mucous membrane of the small intestine with a mucofibrinous layer and whitish-yellowish nodules scattered throughout the intestines that are also visible from the serous side, and less often hemorrhagic enteritis.

The genus *Cryptospridium* has long been considered a commissural stain, to establish that it represents a dangerous zoonotic pathogen. Cryptospores show monoxy according to the vertebrate class in which the host is found, so that interspecies infection is possible only to that extent. Cryptospore oocysts are small and spherical, 5-7 micrometers in diameter, without micropyle and polar cap. The oocyst wall is surrounded by a thick membrane and is colorless. Sporulated oocysts have 4 sporozoites. The development of the parasite in the host is identical to that of other coccidia, except for the location of the causative agent. Unlike other coccidia that are implanted in the microvilli of the intestine during the schizogonic phase, cryptospores form a parasitophorous vacuole in the microvillus located between the outer membrane and the cytoplasm of the microvilli. The spraying of these vacuoles causes much more serious lesions and damage to the intestinal epithelium than in infections with other types of coccidia. The sexual part (gametogonia) is identical to that of other coccidia, and newly fertilized oocysts continue their development intracytoplasmically before excretion and leave the host as infectious oocysts.

Cryptosporidiosis of small ruminants is caused by *Cryptosporidium parvum, C. hominis* (*previously C. parvum genotype 1*), *C. canis* and *C. felis*. All the mentioned species are zoonotic. Infections are caused by ingestion of oocysts through contaminated food and water. The development of the parasite in the host is identical to that of other coccidia, except for the location of the causative agent. Unlike other coccidia that are implanted in the microvilli of the intestine during the schizogonic phase, cryptospores form a parasitophorous vacuole in the microvillus located between the outer membrane and the cytoplasm of the microvilli.

The spraying of these vacuoles causes much more serious lesions and damage to the intestinal epithelium than in infections with other types of coccidia. The sexual part (gametogonia) is identical to that of other coccidia, and newly fertilized oocysts continue their development intracytoplasmically before excretion and leave the host as infectious oocysts. The disease occurs clinically only in animals at the age of 4-10 days and is characterized by a large number of infected animals and considerable mortality.

Symptoms of acute cryptosporidiosis are loss of appetite and weight loss. In the clinical picture, there is yellow-brown watery diarrhea with an unpleasant smell, and sometimes blood is also present. Abdominal pain occurs, sometimes anemia, loss of appetite, dehydration, tenismus, weakness and weight loss. Depression and inactivity occur.

Pathological changes are present in the small intestines in the form of thickening, edema, hyperemia of the mucosa of the cecum and colon. In addition, there may be numerous hemorrhages and the finding of a dark brown mucofibrinous layer. These pathological changes lead to difficult resorption of nutrients, but also to the clinical manifestation of the disease. As a consequence of cryptosporidiosis, there is a significant decrease in the growth of kids, their lower vitality and weaker progress.

2. PRESENCE OF THIS SHEEP COCCIDIOSIS IN THE WORLD

Infections with protozoa from the genus Eimeria and Cryptospridium are the most widespread and we have data on them in research from Australia, Africa, North and South America, Asia and Europe. Numerous data from world literature support the fact that coccidiosis is the most common parasitic disease of sheep in herds. All over the world, an extremely large number of older animals are infected with protozoa, which are involved in the etiology of this disease. It is of even greater importance that the infection of the young that occurs already in the first months of life.

At the same time, adults are the main contaminant of herds and are responsible for maintaining parasitic infections in herds. Their presence has been noted equally in the most modern, hygienically impeccable cultivation conditions as well as in extensive keeping. In the foreign literature, there are extremely numerous data on the study of protozoan infections of sheep and goats, and they all confirm this claim.

Based on existing data, the prevalence of coccidiosis is highest in environments where the most intensive keeping is in stable conditions. In contrast to helminths, which need free grazing areas for biological development, protozoan infections are related to animal husbandry where there is a permanent microclimate, especially temperature and humidity. In addition, a large number of animals of different ages in a relatively small area leads to population pressure that favors the development of protozoa and thus infections.

In countries with extremely developed sheep farming (China, India, Australia, Nigeria, UK, Greece), coccidia represent a major health and economic problem, as well as in third world countries. That's why program control measures with coccidiostat treatments are applied there. It is based on drug therapy and prevention that we present here

- Treatment of coccidiosis caused by Eimeria sp. -

Sulfaquinoxaline in drinking water (concentration 0.015% for 3-5 days) can be used to treat affected lambs. Sulfamethazine (sulfadimidine) can be administered at a dose of 247.5 mg/kg on the first day, then 124 mg/kg for 3 days. Sulfadimethoxine is administered at a dose of 55 mg/kg on the first day, then 27.5 mg/kg for 3 days, orally or in drinking water. Because water intake is often reduced, sick animals should be medicated by feeding or drenching.

Triazinon preparations belonging to the benzene acetonitrile group: diclazuril (1 mg/kg, PO, once or repeated 3 weeks later) is licensed in some countries without a withdrawal period and the preparation Toltrazuril (20 mg/kg, PO, once) used to reduce the number of oocysts. Ponazuril, a metabolite of toltrazuril, is also suggested for the control of coccidiosis in sheep.

Decoquinate (1 mg/kg, PO in feed, every 24 hours for 28 days) is licensed in some countries, including the USA, for young, non-lactating ewes; it is mixed into the medicated meal at the recommended 100 mg/kg of feed, which provides 100 g of feed for a 10 kg lamb for 28 days. Decoquinate can prevent reduced neutrophil function due to parasites.

Amprolium (50–55 mg/kg, PO, every 24 hours for 5 days) has proven efficacy but is not registered for sheep, so its use is not always completely acceptable in some countries (eng. off label use) and should be under veterinary control. Use in lambs is controversial because it can cause thiamine deficiency and related neurological diseases. As a precaution, thiamine (vitamin B1) is also often given after treatment.

- Prevention of coccidiosis –

The protocols used in the world define the combination of monensin and lasalocid (in a dose of 22 and 100 mg/kg of food respectively) which provide an effective prophylactic effect against coccidiosis that occurs in early weaned lambs in feedlot conditions.

Monensin is used in the USA as a component of a concentrate ration at 15 g/ton and is given to ewes from 4 weeks pre-lambing until weaning, and to lambs from 4-20 weeks of age. The toxicity level for lambs is 4 mg/kg, while for goats in the US it is only approved for stable conditions, not for grazing animals.

Lasalocid (15–70 mg per sheep per day, depending on body weight) alone or in combination with monensin and lasalocid (dose: from 22 or 100 mg/kg feed), is also used prophylactically against coccidiosis in early weaned lambs in farm conditions.

Feed decoquinate is approved in many countries for the control of coccidia in feed lambs (1 mg/kg, PO feed, every 24 hours for 28 days). It is also effective in reducing oocyst shedding when sheep are continuously fed dry feed at 0.5 mg/kg (use of 60 g/kg premix at 833 g/ton feed provides a recommended treatment dose of 50 mg/kg feed). Duration of therapy of at least 28 days should be ensured.

Toltrazuril (20 mg/kg, PO, once) is approved for use in many countries prophylactically, before expected shedding of oocysts. Diclazuril (1 mg/kg, PO, once, with a possible second dose 3 weeks later) is also permitted in some countries for sheep aged 4–6 weeks. The doses used are also therapeutic, and the effectiveness of these preparations and their combinations has been proven on several animal species.

- Treatment of coccidiosis caused by *Cryptosporidium* sp.-

As no specific ovine treatment measures are available at present, only the symptoms can be treated, by keeping lambs warm and giving rehydration therapy. However, in some cases, it is possible to use calf treatments, such as Halocur (Halofuginone lactate) off-licence under the Veterinary Medicines Directorate cascade, speak to your veterinarian for further information.

- Prevention of Cryptosporidiosis –

In the face of an outbreak of cryptosporidiosis in lambs there is little that can be done other than to isolate affected stock, avoiding overcrowding or prolonged use of contaminated areas. The tiny, fragile looking oocysts are in fact remarkably tough, and can survive in the environment for many months. Disinfection can be achieved by steam heat or by using Oo-cide (Antec International Ltd.), an ammonia-based oocysticide, which is particularly effective against Cryptosporidium oocysts.

Many treatment plants that take raw water from rivers, lakes, and reservoirs for public drinking water production use conventional filtration technologies. Direct filtration, which is typically used to treat water with low particulate levels, includes coagulation and filtration but not sedimentation. Other common filtration processes including slow sand filters, diatomaceous earth filters, and membranes will remove 99% of Cryptosporidium membranes and bag- and cartridge-filter products remove Cryptosporidium specifically.

Cryptosporidium is highly resistant to chlorine disinfection; but with high enough concentrations and contact time, Cryptosporidium inactivation will occur with chlorine dioxide and ozone treatment. In general, the required levels of chlorine preclude the use of chlorine disinfection as a reliable method to control Cryptosporidium in drinking water. Ultraviolet light treatment at relatively low doses will inactivate Cryptosporidium

3. PRESENCE OF SHEEP COCCIDIOSIS IN SERBIA

Research on the intestinal protozoan fauna of sheep in Serbia was carried out in the mid-fifties of the last century and was primarily of a faunal nature, with monitoring of prevalence. Then there was a slightly longer time break (of about half a century) so that there are almost no valid data from the earlier period.

The continuation of these researches began at the end of the nineties. In the 2000s, several projects of the Ministry of Education, Science and Technological Development were launched. Thanks to them, research on the parasitic fauna of sheep and goats gained full momentum. In twenty years, herds of small ruminants from Vojvodina to Northern Kosovo were covered. Biodiversity and seasonal dynamics of ectoparasites (primarily ticks), helminths and protozoa in small ruminants were determined.

In the mentioned period, it was determined that the dominant infection of small ruminants was caused by coccidia from the genus Eimeria, which ranged from 26.31 to 39.78%. Cryptosporidiosis was observed in the range from 3.22% to 4.90%.

The influence of parasitic infections on the production results and economic aspects of these infections were investigated. New testing methods were developed and modified, some of which were later protected by the Intellectual Property Office of the Republic of Serbia. During these 20 years of research, biosecurity measures necessary for high-quality and profitable production were developed.

Based on these findings, in addition to the primary suppression of parasites in individual herds, measures were also developed for the preventive rehabilitation of pastures and the development of prophylaxis measures and improvement of the keeping conditions of sheep and goats as a primary task. All this was accompanied by published works, with several monographs and works presented at international and national meetings.

4. COMPREHENSIVE PROGRAM FOR THE CONTROL OF COCCIDIOSIS IN SHEEP

The main goal of the comprehensive program for the control of coccidiosis in sheep is to raise the health status of sheep in the Republic of Serbia. By preventing the occurrence and spread of coccidiosis by undertaking certain biotechnical and health measures that have the role of reducing the prevalence of parasites, higher growth and better production results of sheep are achieved. The application of this integrated concept of parasite infection control required systematic monitoring of infection on farms, before and after the applied measures, and required the involvement of all relevant subjects, primarily the veterinary service, starting from farms to animal workers.

The coccidiosis control program was experimentally carried out and applied to nine test farms that raise sheep in herds with more than 50 animals, starting from 2014 to 2018. After the evolution of rejected results, it became a vital zootechnical measure on those farms.

In order to establish the prevalence of parasitic infections and the biodiversity of the causative agent in the investigated sheep herds, samples of feces of all categories of animals were collected. On each of the farms, 30 samples of feces were sampled: 10 from each production category - lambs/kids at teats (from 0 to 28 days of age), rearing animals (before the first release) and breeding animals.

Collected faeces samples were processed by usual parasitological examination methods. We performed examinations for parasites both during the necropsy of dead animals and on the slaughter line. All applied diagnostic methods were in accordance with WFP-EFP, WHO, FAO and OIE recommendations. All parasites were determined on the basis of morphological characteristics, according to the keys given by Soulsby.

On the basis of the obtained findings, the application of a number of solutions was started, which as a whole were incorporated into a single unit whose goal is the control of parasitic infections on pasture and in pens and finally their eradication.

Based on the place of application of individual segments of this technological solution, they can be grouped into two basic units a) measures taken in pens and b) measures taken on pasture.

5. MEASURES TAKEN ON FARMS

In herds in which the presence of parasitic infections was determined or for the purpose of regular control for the presence of parasites, the following measures were applied:

5.1. STRICT OBSERVANCE OF BIOSAFETY AND ZOO TECHNICAL MEASURES

Biosecurity measures aim to improve the profitability of production, prevent unwanted situations and enable effective prevention of infectious diseases. These are general measures related to the correct choice of location for the construction of facilities, the structure and functionality of the facility and infrastructure on farms, as well as zootechnical measures in the production chain. They include the selection of the grazing site as well as the holding technology.

Apart from these measures, biosecurity measures related to the health control of newly acquired animals and prevention of contact between different age categories are also very important.

Internal biosecurity measures are defined in biosecurity protocols on farms, through sanitation plans that are continuously implemented. A sanitary procedure plan is made individually for each facility, depending on the facility's capacity, construction and technical characteristics, and type of production.

Zootechnical measures and their proper application are the next measure in preventing the introduction and spread of infection in herds. As part of this program, an inter-round break, i.e. the so-called "facility rest". Appropriate cleaning equipment was used in each facility, which was not allowed to be used in other facilities.

In cases of persistent infection, a system of daily fertilization is applied with adequate transportation and disposal of manure.

5.2. PRODUCTION PROCESS MANAGEMENT

Sheep are raised in farm conditions during the winter and on pasture during most of the year. For these reasons, attention must be paid to each of the mentioned aspects of this production. The most important moment for the occurrence of coccidiosis is the keeping of sheep in farm facilities.

5.3. PRODUCTION PROCESS ON SHEEP FARMS

5.3.1. Construction and types of objects

Building a sheep farm means meeting the conditions and regulations stipulated by the law, on the one hand, but also creating opportunities for the proper implementation of all production processes, which result from the work program, on the other hand.

Since a sheep farm in the narrower sense means facilities and equipment, to this end it is necessary that each farm includes the following:

- Facility for accommodation of the planned number of sheep with appropriate young, as well as for breeding males;
- Facilities for milking, reception and processing of milk if it is a milk production farm;

- Facilities for housing animal feed;
- Facilities for machines and mechanization;
- Adequate equipment in built facilities.

The sheep facility must provide good living conditions for all categories and the smooth performance of all work processes, from insemination of sheep, monitoring of pregnant ewe, reduction of losses during lambing, rearing of young, to nutrition, selection, milking, shearing and implementation of other measures on which success in sustainable sheep farming depends.

Starting from the mentioned principles, when building a sheepfold, it is necessary to comply with the following criteria:

- The building must face southeast or southwest;
- The location should be in a dry and draining place, sheltered from strong winds;
- Proper lighting through windows, as well as with electricity;
- The temperature in the facility during the winter should be around 6°C for adult animals, and for young 10-12°C; and in the summer months it must not be above 27°C;
- Relative air humidity should range from 80-70%;

5.3.2. General hygiene measures

General hygiene measures are carried out regularly before entering and after leaving the animals from the facilities on the farm. They begin with mechanical cleaning, which aims to remove visible dirt from all surfaces in the facility. When cleaning, it is necessary to wet the surface beforehand so that no dust is raised during cleaning. After finishing the cleaning of the building, all roads inside the economic yard of the farm are cleaned.

The next stage is sanitary washing, which removes the rest of the dirt, and this is done with water whose temperature exceeds 60oC. Sanitary washing should be carried out particularly thoroughly on floors, lower parts of walls and boxes in stables, work surfaces, etc.

5.3.3. Disinfection

Disinfection of buildings is divided into ongoing disinfection, disinfection that is done after eviction and before re-occupation of buildings and sanitary disinfection that is carried out in the event of an outbreak of disease on the farm.

Disinfection is carried out with mildly toxic and non-corrosive disinfectants (most often quaternary ammoniums), which are used to treat equipment in the facility and milking equipment.

In case of disease, sanitary disinfection is applied. In doing so, the sick animals are separated from the healthy ones and both groups are moved out of the facility. After general hygiene measures, disinfection is carried out. Ammonia-based disinfectants destroy coccidia oocysts and are used for floor mats (straw). Other surfaces are treated with a 2% solution of NaOH in warm water of at least 70°C.

5.3.4. Rodentization and disinsection

Rodent control is one of the basic measures of zoohygiene protocols. In addition to the application of chemical rodenticides, it is good if construction and technical measures are taken in order to reduce the entry of rodents into farm buildings.

Disinsection of buildings should be carried out continuously, bearing in mind the presence of a large population of insects, primarily flies, in the buildings themselves and in the immediate surroundings. Hygienic and protective measures should be used on the farm, first of all, proper storage of manure, installation of protective nets on windows and ventilation openings, insect traps, electric UV electrocutor lamps, and, if necessary, the use of chemical agents (adulticides and larvicides).

5.3.5. Control of birds and stray dogs and cats

Control of birds found on farms and on individual holdings with a large number of animals (pigeons, sparrows, starlings, swallows, crows, magpies, etc.) must be rigorously implemented, considering that they can be carriers of infectious agents (tuberculosis, salmonellosis, ornithosis, pasterelosis etc.). Therefore, it is recommended to install nets on windows and ventilation openings, close silo openings and remove places suitable for making nests and holding birds, as well as spikes to prevent birds from landing. Chemical treatment with corvicide is the option of last resort.

The presence of stray animals, dogs and cats, is a frequent occurrence on a large number of farms. These animal species should be denied access to farm facilities, considering that dogs are carriers of many zoonotic parasites, most often tapeworms, the most important of which are *Echinoccocus granulosus, Taenia multiceps, T. hydatigena, T. serialis* and others whose cysts are found in the organs or brains of infected sheep, and certain species such as E. granulosus and *T. multiceps* are zoonotic parasites. Dogs can also transmit C*ryptosporidium* sp. to sheep and *Giardia intestinalis* which are zoonotic protozoa.Cats are carriers of toxoplasmosis, one of the most pathogenic protozoan zoonoses *Toxoplasma gondii* that affect mammals and birds, and human infections are caused by the consumption of unheated meat and milk from infected animals.

5.3.6. Removal of dead animal carcasses

Removal of carcasses of dead animals from production facilities is the responsibility of employed workers. During the construction of the farm, space is planned for burial pits and an animal cemetery. On better farms, there are built cooling chambers or containers for collecting carcasses from the farm, i.e. all materials that belong to category 1. Their removal is carried out according to a weekly plan, and it is better to process the carcasses in rendering plants, where it is necessary for each farm to have a signed contract with the rendering plant for the removal of category 1 materials. In this way, the risk that the corpses of dead animals are a source of various infections for healthy sheep is reduced.

5.3.7. Removal of manure

The sheep are kept on a deep mat in the barns. In such facilities, manure is formed on the entire surface of the floor and is usually removed twice a year. Manure disposal within the farm must be in drained places, at least 50 m away from buildings. Application of biological degradation of manure (burning of manure) is considered desirable and is very important for raising the level of biosecurity on the farm. The use of manure originating from small ruminant farms, for the purposes of fertilizing agricultural areas, represents a significant risk to the health of animals as well as human health if the manure is not adequately treated by lying down. Research in the world and in our country has shown that the presence of *Eimeria* sp., *Cryptosporidium* sp. oocysts can be found in soil and sediment, and various types of pathogenic bacteria including *Salmonella* sp. and *Staphylococcus aureus*.

6. MEASURES TAKEN ON PASTURE

6.1. PASTURE

A pasture is an area of land that is used for feeding and raising livestock by grazing. Depending on the altitude, it can be mountain or lowland pasture. They are important sources of fodder for the production of green mass for grazing and making hay, and more and more for the production of quality silage and haylage, especially in hilly and mountainous areas. Besides the obvious importance of pastures, they are also the site of constant parasitic infections of small ruminants. There are many factors that contribute to the occurrence, maintenance and spread of parasitosis in one pasture. Among them are: joint keeping of animals of different old categories, joint keeping of animals of different owners on the same pastures and, as the most important, favorable climatic conditions that are necessary for the development and survival of preparatory stages and transitional hosts of parasites in the external environment. The conditions required for the development of certain types of parasites vary and are reflected in their seasonal distribution from year to year.

6.2. PROPER USE OF PASTURE

The population pressure on the pasture (number of animals per unit area) and the method of grazing - whether it is forced or stationary also affect the load on the pasture and the degree of its infection. That is why one of the solutions that is successfully used in the form of grazing - with this type of infection, the best solution is persecution grazing.

When lambs are kept on grass, these areas must not be overcrowded and they need watering places located in well-drained areas. Shelters must be kept clean and have dry, well-laid floors. The grass in the pens should be kept for a long time in order for the kid/kid to develop immunity through low exposure to coccidia oocysts.

Peregrine grazing is a method of using pastures in which animals are moved from one part of the pasture to another at intervals and returned to them only after a certain period of rest. Usually, persecutions are carried out after 5-7 days, so that in 2-3 months the animals return to the initial location. The cleanest part of the pasture should be used for young animals, which is achieved by grazing them in front of older animals. In some places, this is solved by making movable fences that can also be used to block places where grazing is not desirable (next to ponds, etc.).

6.3. ERADICATION OF PASTURES

Permanent pastures pose the greatest health risk for sheep, especially if they have been used unplanned for many years. The cultivation of pastures directly depends on the geological and pedological composition of the soil, hydrological conditions (standing and running water) and microclimatic conditions. The main goal of cultivation is to obtain a pasture that contains a minimum of infectious agents in the soil, which is maximally free from vectors of certain diseases (molluscs, arthropods), infectious forms of parasites and other infectious agents that can be found on the grass.

The earlier opinion that infectious agents are destroyed by plowing permanent pastures is refuted by the fact that in this way (ploughing) certain parasites are only successfully protected from unfavorable external conditions - direct insolation, drying, showers, frost, etc. Without the use of certain disinfectants and artificial fertilization, the primary effect of this measure is short-lived. Any correction of the pasture after leveling the quantity and quality of the soil is carried out through the floristic composition of the plant mass and the application of manure, which must be adequately prepared.

7. HEALTH PROTECTION

By making an analogy with the health condition of small ruminants in the surrounding countries, where this production is on the rise, an indicator of the epizootiological and epidemiological situation can be extracted, which has not been analyzed in detail until now. Knowledge of the correlation between microclimatic conditions and disease vectors - high-altitude pastures, the presence of floodplains, the absence of any land reclamation and cultivation of pastures, etc. indicates the presence of several diseases that we will list here. Most of them have a zoonotic character, so they represent a serious health problem in case of occurrence.

By looking at the epizootic situation and the movement of infectious and parasitic diseases of sheep in our country, it is possible to single out diseases of viral, bacterial and parasitic etiology that are most often present and the control measures required for their suppression and control.

7.1. PARASITOLOGICAL DIAGNOSTICS

Regular parasitological examinations must be included in regular preventive health care measures for sheep. Timely detection of parasitic infections and targeted therapy are the basic measure of successful suppression of parasitic infections both on pasture and during the winter stay in the pens, and thus the achievement of better production results.

Preventive coprological diagnostics should include all age categories of animals and is performed at least three times a year in all age and production categories. Breeding animals, if they are positive, should be dewormed and treated with acaricides before conception and, if necessary, before parturition.

We must perform a parasitological examination of the decided lambs before taking them out to pasture and treat them if necessary. For fattening animals and breeding cows that are on pasture and in pens, the same principles apply - regular and periodic controls. Also, newly acquired breeding animals must undergo parasitological control twice during their stay in quarantine.

Coprological examinations in sheep are performed using the methods of Patakij, Berman, McMaster, as well as the modified method of Whitlook. To assess the degree of infection, we used the subjective method of descriptive description according to McMaster. Determination of oocysts is done morphometrically according to the keys given by Soulsby.

In all cases of a positive finding, parasite control must be carried out in all animals.

7.2. APPLICATION OF ANTIPARASITIC DRUGS

During drug therapy, we expel the parasites from the animal's digestive tract, so after the therapy, which is carried out in the pens, we have to remove the parasites together with the garbage. Antiparasitics are administered through food and water.

- **Coccidiosis therapy**

In our experiment, after diagnosing parasitic infections with Eimeria sp. all animals in the herd were treated with Toltrazuril-la (20 mg/kg, PO, once), whose metabolite is toltrazuril-sulfone (ponazuril), which significantly reduces the number of cysts.

- Coccidiosis prevention

The occurrence of coccidiosis caused by *Eimeria* sp. under many management systems it is predictable, so it may be necessary to administer prophylactic coccidiostats in feed or soluble coccidiostats for 28 consecutive days, starting a few days after lambs are introduced to the suspected environment. However, this does not mean that other appropriate corrective changes in management should not be sought and introduced. Sulfonamides can be added to drinking water as a treatment, but are not FDA approved for this purpose. In our case, it was used for preventive purposes before being driven out to pasture and before returning to the stables *Toltrazuril* (20 mg/kg, PO, one-time).

- Cryptosporidiosis therapy

In case of cryptosporidiosis is no specific ovine treatment measures are available at present, only the symptoms can be treated, by keeping lambs warm and giving rehydration therapy. The application of *Toltrazuril* (20 mg/kg, PO, one-time).also showed a satisfactory effect.

- Cryptosporidium prevention

In general, the required levels of chlorine preclude the use of chlorine disinfection as a reliable method to control Cryptosporidium in drinking water.

7.3. HYGIENE MEASURES DURING AND AFTER THERAPY

All clinically affected lambs should be separated from their mothers and placed in a clean, disinfected pen with plenty of straw. Other animals should be moved to less contaminated areas of the facility to ensure that food and water sources are free of feces.

Controlling the management of coccidiosis is a delicate balance between ensuring that lambs are exposed to the infection and thus acquire immunity and preventing their development and causing the onset of clinical disease. Management plays a key role in prevention by ensuring that all areas used by sheep, especially lambs, are thoroughly cleaned and disinfected, that pens are not overcrowded, and that kids are kept in small groups of the same age. The number of individuals in the facility is reduced, and thus fecal contamination. All feed and water troughs should be raised off the ground and placed in well-drained areas. By regularly moving the lambs to other segments of the facility, excessive accumulation of oocysts is prevented and the potential infectious dose is reduced.

8. EDUCATIVE PROGRAM

The training of veterinarians who provide health care for small ruminants was carried out during each visit to the stations, as part of the contractual cooperation. Special emphasis is placed on the application of good veterinary practice. In this way, a contribution was made to preventing the spread of infection, but also educated veterinarians on how to protect themselves from balantidiosis and cryptosporidiosis, which in some countries have the status of professional diseases.

9. RESULTS OF TAKEN MEASURES AND CONCLUDING CONSIDERATIONS

For the purpose of quantifying coccidiosis control measures applied to flocks of sheep in pens and on pasture, which were implemented starting from 2014 to 2018, and were carried out by the competent veterinary stations, parasitological examinations of the flock were carried out.

9.1. CONTROL PARASITOLOGICAL EXAMINATIONS OF THE HERD

- Number and type of sample

In order to establish the prevalence of coccidiosis and the biodiversity of the causative agent in the studied herds of sheep and goats, samples of feces of all categories of animals were collected. On each of the farms, 30 samples of feces were sampled, 10 from each production category - suckling lambs (from 0 to 28 days of age), rearing animals (before the first mating) and adult sheep (breeding animals).

-Methods of examination

Coprological examinations were performed using the methods of Patakij, Stoll and McMaster as well as the modified method of Whitlook and Euzeby. To assess the infection, we used the subjective method of descriptive description according to McMaster, in accordance with the recommendations of WFP-EFP, WHO, FAO and OIE. All parasites were determined on the basis of morphological characteristics, according to the keys given by Soulsby.

- Intervals of examination

1. The first control parasitological examination was carried out in the winter months during the stay of animals in closed buildings, when coccidiosis occurs most often.
2. The second examination was performed after the application of preventive therapy before the expulsion to the pasture.
3. The third inspection was done three months later during the grazing season.
4. The fourth inspection was done before the withdrawal of the animals into the pens when the second preventive treatment was done.
5. The fifth parasitological examination was performed after one month of the animals' stay in the stables.

9.2. RESULTS OF CONDUCTED RESEARCH

At the beginning of the implementation of the program, the prevalence of parasitic infections in the tested herds was as follows:

Our first examination revealed coccidiosis in 139 animals (23.61%). Infection was most common in lambs (less than 3 months old), sheep aged 3 months to 1 year, and adult sheep (1 year or older).

Lambs most often showed clinical symptoms of the disease and they were present in 39.37% of the affected individuals. There was no gender difference in the prevalence and incidence of coccidiosis.

In sheep between the ages of 3 months and 1 year, rearing animals (before the first admission) 51.47% were infected with coccidia in a subclinical scale, with the fact that there was no gender difference in the prevalence and incidence of coccidiosis.

In adult animals, the results showed that 57.70% of sheep were infected and had subclinical coccidiosis. Adult female sheep were significantly ($p<0.05$) more infected (82.2%) than adult rams (40%).

The most abundant species of Eimeria in lambs was *E. ovolidalis*, while in sheep aged from 3 months to 1 year and adult sheep, *E. ovina* was the most common.

In the overall findings, *E. ovoidalis* was the most abundant species, found with 87.1%, followed by *E. faueri* (63%), *E. ovina* (53%), *E. granulosa* (31%), *E. parva*, (25%), *E. intricata* (19%) and *E. pallida* (8%), Infections with two or more Eimeria species were detected in 78%.

- First parasitological examination

The total number of excreted oocysts in lambs was 95763+/-61283 OPG, while in sheep aged from 3 months to 1 year it was 34951+/-33237 OPG and in adult sheep 39799+/-31117 OPG. This is explained by the acquired immunity of sheep that arises after each infection, and that is why in adult animals we have a rarely manifested clinical picture of the disease, but they are constant transmitters of the infection to younger animals.

All animals were treated with a dose of Toltrazuril (20 mg/kg, PO, once).

- The second parasitological examination after the application of preventive therapy before the expulsion to the pasture

The total number of excreted oocysts in lambs was 58827+/-45675 oocysts per gram of faeces (OPG), while in sheep aged 3 months to 1 year it was 23961+/-21019 OPG and in adult sheep 19015+/-16989 OPG.

All animals were treated with a dose of Toltrazuril (20 mg/kg, PO, once)..

- The third parasitological examination was done three months later during the grazing season

The total number of excreted oocysts in lambs was 69898+/-51778 OPG, while in sheep aged from 3 months to 1 year it was 37654+/-33458 OPG and in adult sheep 21674+/-18678 OPG.

- The fourth parasitological examination before withdrawing the animals to the pens after the second treatment

Before retiring to the barn, all animals received preventive treatment with Toltrazuril (20 mg/kg, PO, once).

The total number of excreted oocysts in lambs was 12594+/- 11118 OPG, while in sheep aged from 3 months to 1 year it was 11256+/-10927 OPG and in adult sheep 9796+/-8217 OPG.

- The fifth parasitological examination after a month of the animals staying in the barn

The total number of excreted oocysts in lambs was 11892+/- 10584 OPG, while in sheep aged 3 months to 1 year it was 10017+/-9747 OPG and in adult sheep 9799+/-8217 OPG.

Tables 1, 2 and 3 show a reduction in the prevalence of coccidiosis after the application of the mentioned technical solution. This is illustrated graphically in picture 1, 2 and 3.

The tables show a reduction in the prevalence of coccidiosis after the application of the mentioned technical solution, and after its application we no longer had the occurrence of clinically manifest coccidiosis that was present in the lambs during the first examination, before the application of the therapy.

Based on the conducted tests, we believe that by applying comprehensive measures, starting with the measures applied in the pens on the farm, through the measures applied on the pasture, in a relatively short time they achieved a significant contribution in controlling and reducing the prevalence of coccidiosis in sheep.

Table 1. Prevalence of coccidiosis in suckling lambs (from 0 to 28 days of age) during the research period.

examination	number of oocysts per gram of faeces (OPG)		
	min	max	average
1	61283	95763	78533
2	45675	58827	52251
3	51778	69898	60838
4	11118	12594	11856
5	10584	10892	10738

Picture 1. Prevalence of coccidiosis in suckling lambs (from 0 to 28 days of age) during the research period

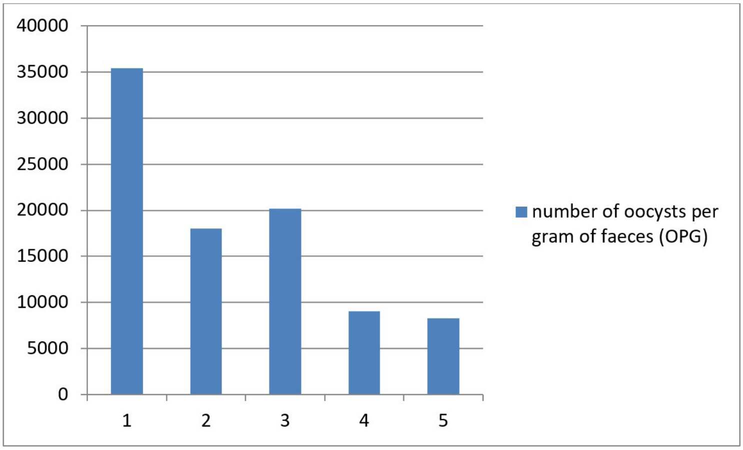


Table 2. Prevalence of coccidiosis in sheep aged from 3 months to 1 year of rearing animals during the research period.

examination	number of oocysts per gram of faeces (OPG)		
	min	max	average
1	33237	34951	34112
2	21019	23961	22490
3	33458	37654	35556
4	11928	10256	11092
5	9747	10017	9882

Picture 2. Prevalence of coccidiosis in sheep aged from 3 months to 1 year of rearing animals during the research period.

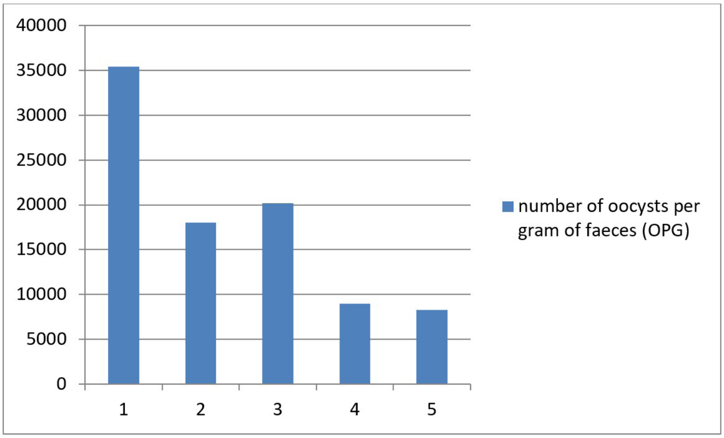
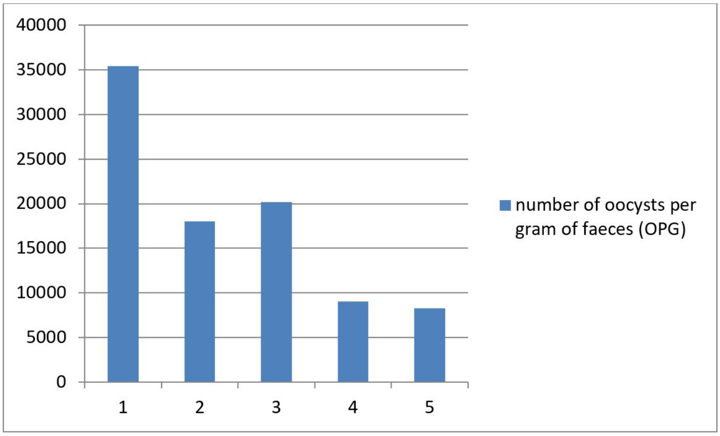


Table 3. Prevalence of coccidiosis in adult (breeding) sheep during the research period.

examination	number of oocysts per gram of faeces (OPG)		
	min	max	average
1	31117	39799	35458
2	16989	19015	18002
3	21674	18678	20176
4	9799	8217	9008
5	7738	8974	8266

Picrure 3. Prevalence of coccidiosis in adult (breeding) sheep during the research period.



These measures achieved the goal of controlling coccidiosis in sheep/lambs in two ways a) by creating primary immunity in lambs after subclinical infection, which achieves lifelong immunization that prevents clinically manifest diseases and b) by preventive therapy that is used at the moment when, due to population pressure and environmental conditions, predisposing conditions for immune breakthrough and the emergence of clinically manifest infections have been created. This refers primarily to the winter period, when a critical amount of oocysts per unit area is reached in the pens and the possibility of infection. Another critical point is the stay on pasture when, due to contamination of the soil, the possibility of pasture infections is created, which was also brought under control with this program.

By cutting these points of infection, with adequate zootechnical, zoohygienic and therapeutic means, the development of natural immunity is achieved and allows coccidiosis not to appear as an obligatory infection, but to turn into a commissural infection whose presence is constantly kept under control.

Based on the obtained findings, the applied measures became part of the regular program of sheep health care in the examined herds. A special contribution to the implementation of these measures was made by fellow veterinarians, and the results achieved were a satisfaction both for them and for us, who conceived and implemented this complex coccidiosis control program.

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SOBRE O ORGANIZADOR

EDUARDO EUGENIO SPERS realizou pós-doutorado na Wageningen University (WUR), Holanda, e especialização no IGIA, França. Possui doutorado em Administração pela Universidade de São Paulo (USP). Foi Professor do Programa de Mestrado e Doutorado em Administração e do Mestrado Profissional em Comportamento do Consumidor da ESPM. Líder do tema Teoria, Epistemologia e Métodos de Pesquisa em Marketing na Associação Nacional de Pós-Graduação e Pesquisa em Administração (ANPAD). Participou de diversos projetos de consultoria e pesquisa coordenados pelo PENSA e Markestrat. É Professor Titular no Departamento de Economia, Administração e Sociologia, docente do Mestrado em Administração e Coordenador do Grupo de Extensão MarkEsalq no campus da USP/Esalq. Proferiu palestras em diversos eventos acadêmicos e profissionais, com diversos artigos publicados em periódicos nacionais e internacionais, livros e capítulos de livros sobre agronegócios, com foco no marketing e no comportamento do produtor rural e do consumidor de alimentos.

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