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EPIDEMIOLOGY AND CONTROL OF GASTROINTESTINAL NEMATODES IN LITHUANIAN GOAT FARMS

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<th>Description</th>
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<tbody>
<tr>
<td>ABZ</td>
<td>Albendazole</td>
</tr>
<tr>
<td>BCS</td>
<td>Body Condition Score</td>
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<tr>
<td>BZ</td>
<td>Benzimidazoles</td>
</tr>
<tr>
<td>CI 95%</td>
<td>confidence limit of the mean</td>
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<tr>
<td>DL₄</td>
<td>Development stage larvae of L₄</td>
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<tr>
<td>EHA</td>
<td>Egg Hatch Assay</td>
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<tr>
<td>EL₄</td>
<td>Early stage of larvae L₄</td>
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<tr>
<td>EPG</td>
<td>Eggs Per Gram faeces</td>
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<tr>
<td>FBZ</td>
<td>Fenbendazole</td>
</tr>
<tr>
<td>FEC</td>
<td>Faecal Egg Count</td>
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<tr>
<td>FECR</td>
<td>Faecal Egg Count Reduction [%]</td>
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<tr>
<td>FECRT</td>
<td>Faecal Egg Count Reduction Test</td>
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<tr>
<td>GIN</td>
<td>Gastrointestinal Nematodes</td>
</tr>
<tr>
<td>HIG</td>
<td>High Intensity Grazing farm</td>
</tr>
<tr>
<td>IVM</td>
<td>Ivermectin</td>
</tr>
<tr>
<td>L₁</td>
<td>First stage larvae</td>
</tr>
<tr>
<td>L₂</td>
<td>Second stage larvae</td>
</tr>
<tr>
<td>L₃</td>
<td>Third stage larvae</td>
</tr>
<tr>
<td>L₄</td>
<td>Fourth stage larvae</td>
</tr>
<tr>
<td>LD 50</td>
<td>Lethal Dose, 50%</td>
</tr>
<tr>
<td>LD 99</td>
<td>Lethal Dose, 99%</td>
</tr>
<tr>
<td>LEV</td>
<td>Levamisole</td>
</tr>
<tr>
<td>LIG</td>
<td>Low Intensity Grazing farm</td>
</tr>
<tr>
<td>MALDT</td>
<td>Micro-agar Larval Development Test</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>ML</td>
<td>Macrocyclic Lactone</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>TST</td>
<td>Targeted Selective Treatment</td>
</tr>
<tr>
<td>WAAVP</td>
<td>World Association for the Advancement of Veterinary Parasitology</td>
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INTRODUCTION

Since the conversion from extensive to intensive agriculture, animal health problems have developed with alarming speed. In large scale sheep and goat farming systems endoparasites have become a major threat [30]. In Lithuania, small ruminant farming has been in progress for the last 5 years. The number of goat population increased by 24% and the number of registered goats in 2014 was 9.3 thou in 2014 [1]. Infections with gastrointestinal nematodes can have a detrimental effect on animal health, leading to clinical and sub-clinical diseases which may result in financial loss and overall decrease of productivity [115]. However, reduced productivity and death losses, particularly of kids, due to infection with the blood-feeding abomasal nematode Haemonchus contortus is a serious constraint to economic goat production. It has been anticipated that climatic conditions due to global warming will increase parasite-related losses in grazing livestock [103, 152]. However, it is now apparent that H. contortus is becoming more important in the temperate regions of the world, with the apparent change in weather conditions that favour this parasite [166]. The increasing number of reports on H. contortus in Scandinavian small ruminants [37, 89], is just one example of an epidemiological peculiarity that may be attributed to an on-going climate change. The average temperatures and events of rainfalls have also have increased in Lithuania as a result off global warming [16].

The feeding behaviour and diet of sheep and goats is different [106]. Despite that the majority of nematodes, cestodes and trematodes infecting sheep and goats belong to the same species, these animals have different immune response against parasites [63]. Goats are more susceptible to gastrointestinal nematodes infection than sheep. The study of Huntley et al. [68] confirmed the cellular responses which determine this difference. In addition, pharmacokinetics and efficacy of anthelmintics also represent an important difference between sheep and goats [20, 39, and 122]. Regarding the accelerated metabolism of drugs in goats, the doses have to be doubled to reach higher plasma levels. The farmers and veterinarians often are not informed regarding specificity of anthelmintic usage which leads to mistakes in control of GIN in goats. Furthermore, the knowledge about worm control practices on small ruminant farms shows increments of risk for development of anthelmintic resistance (AR). The surveys based on questionnaires regarding worm control practices in small ruminants were performed in Norway [36], Denmark [92, 93], Slovak Republic [17], France [56] and Spain [111, 119]. It was shown, that high frequency of anthelmintic
usage with small refugium [155], under-dosing and treatment with the same anthelmintic increased AR development [92, 125]. There are reports from different European countries regarding AR in small ruminant farms: in Norway [35], in Sweden [54], in Poland [6], in United Kingdom [10, 99], in Germany/Switzerland [133, 134], in France [23], in Spain [97], in Greece and in Italy [48]. In Lithuania there is no information about AR in small ruminant flocks, however, the study on pig farms showed that the efficacy of ivermectin (IVM) ranged from 90.5% to 100% and of levamisole (LEV) from 84% to 100%, respectively [141]. Furthermore, anthelmintic resistance (FECR) of horse strongyles against fenbendazole (FBZ) with efficacy 86% has been determined whereas pyrantel pamoate was effective with efficacy 99.6% [164].

Previous studies on Lithuanian sheep and cattle farms have shown that both high prevalence of GIN [128] and larval inhibition occur in calves [127]. However, the epidemiological knowledge of GIN of goats is still missing.

Aim and objectives

Aim of the study:

The aim of the present study was to perform the epidemiological survey of trichostrongylid infection in goats, to investigate the prevalence of AR and risk factors associated with AR and to adjust the anthelmintic treatment for control of GI nematodes in grazing goats in Lithuania.

Objectives of the study:

1. To determine seasonal dynamics and intensity of gastrointestinal nematode infections in goats;
2. To determine the risk factors leading to higher gastrointestinal nematode infection;
3. To compare efficacy of strategic control programme on different grazing intensity farms;
4. To explore the faulty control practice, which increases the risk to development of anthelmintic resistance;
5. To assess the anthelmintic resistance status on goat farms in Lithuania.
Scientific novelty and practical significance

For the first time in Lithuania, the prevalence and epidemiology of gastrointestinal (GI) nematodes in goat farms was investigated. According to the highest GI nematodes infection level, the strategic anthelmintic treatment was suggested. In addition, for the first time a survey on anthelmintic resistance (AR) \textit{in vitro} tests was performed, which revealed the actual situation on benzimidazoles, ivermectin and levamisole in goats in Lithuania. These results complemented the knowledge about AR in Europe.

Based on the performed study, a booklet about gastrointestinal parasites infections and control methods for veterinarians and farmers was prepared (Accessory 5).
1. LITERATURE REVIEW

Infections with GINs can cause trichostrongylidosis in small ruminants, depending on the quantity and species of worms present, the general health, the nutritional and immunological status and the age of the animal. The infections occur mostly as mixed infections of different GIN species. There are seven important causative agents of trichostrongylidosis in goats:

Abomasum:
- *Haemonchus* spp.
- *Teladorsagia* spp.

Small intestine:
- *Trichostrongylus* spp.
- *Nematodirus* spp.
- *Cooperia* spp.

Large intestine:
- *Chabertia* sp.
- *Oesophagostomum* spp.

*Teladorsagia* and *Trichostrongylus* spp. are dominant parasites in temperate zone, with cooler climate creating optimum conditions for larval development. This includes regions such as northern Europe, Scandinavia, north Asia, New Zealand and northern North America, where worm infections typically peak in summer or autumn [108]. In Central Europe, several species of the *Teladorsagia* genera occur and of these *Te. circumcincta* and *Te. trifurcata* of *Trichostrongylus* - *T. colubriformis* and *T. vitrinus* are the most important GIN in small ruminants.

*Haemonchus contortus* is the most important nematode species of small ruminant in tropical and subtropical areas, or regions with summer-dominant rainfall, becoming less significant as climate tends towards winter rainfall, and summers become hot and dry [108].

*Chabertia ovina* and *Oesophagostomum venulosum* are the species of highest prevalence in the large intestine of goats in Europe.

### 1.1. Life cycle

Most of gastrointestinal nematodes develop directly and the life cycle has pre-parasitic as well as parasitic stages. The adult worms live in abomasum,
small or large intestine where the male and the female worms mate and produce eggs that are excreted in the faeces (Fig. 1.1.1.1). In the faecal mass, the eggs embryonate and hatch into first-stage (L₁), which in turn moult into second-stage larvae (L₂), shedding their protective cuticle in the process. During this time, the larvae feed on bacteria. The L₂ moult into third-stage larvae (L₃), but retain the cuticle from the previous moult. The L₃ constitute the infective stage, and these are very active, migrate onto surrounding vegetation where they become available for ingestion by grazing animal. The L₃ is attained in about 4 to 6 days after hatching [140]. *Strongyloides* spp. excrete embrionated eggs in faeces. This parasite has two kinds of life cycle, the heterogonic and homogonic [109]. In the homogonic cycle, the nematode egg hatch and moult twice to become the 3rd stage larvae. These larvae infect a host through the skin or are ingested with contaminated pasture, food or water. After ingestion they undertake a migration through blood vessels, lungs, trachea, mouth and small intestine. Infective larvae penetrate the skin and are as well transmitted through milk, infecting newborn ruminants. Other larvae develop indirectly, i.e. they follow a bisexual path (heterogonic cycle) and complete the development to adult males or females in the environment. After mating, adult females produce fertilized eggs that develop to infective L₃ larvae within 7 to 10 days. Once in the host’s gut they complete the development to adults but only females are produced, which begin producing eggs parthenogenetically. Compared to these nematodes the pre-parasitic stage of *Nematodirus* spp., *Trichuris* spp. and *Capillaria* spp. is different in that the development to the infective stage takes place within the egg [149].

The parasitic phase begins when infected larvae are ingested by animals. Following ingestion, the L₃ are shedding in the rumen or intestine, where they penetrate the epithelial layer of the mucus membrane (in the case of *Haemonchus* and *Trichostrongylus*) or enter the gastric glands (*Teladorsagia*). In normal development, the L₃ moult within 2–3 days to become fourth-stage larvae (L₄), which remain in the mucous membrane or in the gastric glands for further 10 to 14 days. Finally, the L₄ emerge and moult to become young adult parasites. The time between ingestion of L₃ and the parasite becoming mature adults (referred to as the prepatent period) varies between parasite species, but generally is between 3 and 5 weeks.

The inhibited stage development is an important aspect of surviving during cold period in temperate regions; particularly of the subfamily *Ostertagiinae* and *Haemonchus contortus*. Its main characteristic is the temporary delay of parasite development in the host. Many explanations have been given of the temporary delay of development of nematodes, for example, host immune response, density-dependent regulatory mechanism
of worm populations, evolutionary adaptation, etc. [3]. Meanwhile, most studies carried out in the Northern hemisphere agree that low temperatures in autumn and early winter are the determinant factor for *Ostertagia ostertagi* inhibition in cattle. The study under laboratory conditions of Fernandez et al. [43] showed the efficacy of low temperature and short light on infective larvae to lead to inhibition development. However, it is concluded that usually there is no single causative factor, but that many factors act together to produce the hypobiosis phenomenon. This phenomenon is important for survival of *H. contortus* in cold conditions, where overwinter survival on pasture is poor. The hypobiotic early L₄ larvae (EL₄) within the abomasal glands begin to accumulate in September in ruminants grazing pastures in cool temperate regions of the northern hemisphere and this accumulation ends when these animals are housed for the winter at the beginning of November. The hypobiosis of *Ostertagia/Teladorsagia* spp. were described in sheep [166], cattle [127] and sika deer [126]. Hypobiosis also occurs in warmer regions, but is not absolute, with wide variations in the proportion of EL₄ involved [148], while in hot climates, hypobiosis is either not observed, or occurs as a parasite survival strategy during seasonal drought periods [46].

![Fig. 1.1.1.1. General life cycle of gastrointestinal nematodes of small ruminants](image)

**Fig. 1.1.1.1. General life cycle of gastrointestinal nematodes of small ruminants**
When environmental conditions become favourable or when host immunity wanes, the parasites complete their development and start producing eggs; this leads to pasture contamination, and therefore represents an important source of infection. The development from inhibited larvae to adult worms in spring may be in synchrony with the beginning of the parturition season, which manifests itself in a peri-parturient increase in faecal egg counts (FECs) in ewes and goats [117]. The peri-parturient decrease of immunity increases the survival and egg production of existing parasites [21], increases susceptibility to further infections, and contributes to the contamination of pasture with L₃s when young, susceptible animals begin grazing. The inhibition of the immune response, specifically associated with gut-dwelling nematodes, is related to serum levels of prolactin. Immune competence is restored when prolactin levels drop, at weaning, and worm burdens are usually expelled as a consequence of the restored immune response. The resumption of parasite activity may also lead to an acute Type II syndrome, resulting in a sudden onset of clinical signs in late winter and early spring [144].

1.1.1. Risk factors for infection

The infection with GIN in the goats depends on the contamination of environment and individual host immunity. The climatic factors of a region (temperature, rainfall, humidity and soil moisture) influence the development and survival in the environment. The development of trichostrongylid larvae occurs at a temperature range of approximately 10–36°C. The optimal humidity requirement for free-living stage development of most species is 85%. Although desiccation is lethal for the free-living stages of parasite worms, the important nematode parasites can survive such conditions either as embryonated eggs or as infective larvae [108]. The L₃ of trichostrongylid nematodes may survive for varying periods, depending on species and particularly the prevailing weather conditions. In the desiccated state L₃ can survive for several months, but once hydrated they become active and rapidly exhaust their food reserves [108, 146]. The study in the Netherlands showed that most of the larvae on pasture without grazing animals not survive longer than 3 months [40]. In general, the combined effects of these factors are responsible for the seasonal fluctuations in the availability of L₃ on pasture, and subsequently in the prevalence of worm burdens in the hosts. Lithuania belongs to temperate climatic zone, where the summers are mid warm and winters are cold with the temperature below 0°C. Our climatic conditions are unfavourable for survival in winter period; however, the infective L₃ larvae may survive on the pasture for the next
Rainfalls and soil moisture are important factors for emergence and spread onto herbage on pasture [2, 151]. Not surprisingly, such associations are most dramatic in semi-arid regions, where absence of rain may bring parasite transmission to a halt during the driest months and sharp peaks in the abundance of larvae on herbage can be seen after periods of rainfall [139]. With respect to migration away from the dung and onto herbage, pasture studies have suggested that a film of moisture, through which larvae would swim, is needed for the migration onto herbage [107]. However, after heavy rains followed by artificial watering of herbage, approximately 85% of larvae were still recovered in the top 5 cm of the soil while only 0.8% reached a depth of 25–30 cm [151]. In Lithuania, favourable conditions to spread the larvae onto herbage during grazing period (April-October) are predetermined by sufficient rainfall, sometimes with short dry periods of the climate.

The stocking rate of goats on pasture is one of the important risk factors. Under high stocking rate the goats decrease feed on pasture what leads to lower nutritional status (lower serum glucose, urea, cholesterol, magnesium and zinc levels), weight loss, lower body condition score (BCS) in the late-wet season, and lower fertility. Goats on the high stocking rate were forced to alter diet selection pattern by consuming more resinous, toxic and coarse species [100]. It is known that the balanced nutrition can enhance the host resistance i.e. its aptitude to regulate the worm populations as well as the host resilience, i.e. its ability to withstand the negative effects of nematode infections [64]. A positive relationship between the stocking rate and pasture infectivity has been shown. The lambs grazing on high stocking rate pasture excreted higher eggs of nematodes [145]. Goats which are allowed to overgraze the pasture are at the greatest risk for developing parasitism because late in the season the parasitic free-living larval populations on the pastures are at their great numbers. In addition, pasture management influence-free living larval population and the infection with gastrointestinal nematodes of goats.

It is well known that the intensity of infection with gastrointestinal nematodes in ruminants is largely dependent on the intensity of host response and its ability to regulate the worm populations. The manifestation of immune response depends on age, nutrition balance and individual (genetic) features. The acquisition of a fully expressed immune response appears on the 12th months of age in goats [63].

A high variability between animals is one of the most striking features of nematode infection of the gastrointestinal tract in small ruminants. Although the information on the subject is less abundant in goats, data also are
available which confirm the occurrence of differences between and within breeds of goat [5, 31, 61 and 162].

1.1.2. Pathophysiological aspects of gastrointestinal nematode infection and immunity

The parasitism in animals causes changes in GI-tract as follows:
- Reduced absorptive area due to loss of mature villi.
- A decrease in absorptive capacity of remaining enterocytes, especially, as many still are immature.
- A reduction in enzyme activities, particularly of brush-border enzymes.
- Reduced contact time between digesta and mucosa [142].

The animals, which were fed with well-balanced forage, are more able to tolerate parasitism than are the animals on a low plane of nutrition [65, 80], however, the morphological and physiological changes emerge in the first days of infection [138]. The abomasal nematodes, of which especially important are Te. circumcincta and H. contortus, inhibit acid secretion by as yet undetermined mechanisms [76]. This in turn reduces pepsinogen activation, increases gastrin secretion, allows microbes to survive, which may reduce their availability as nutrients, and can have marked effects on tissue growth and differentiation [137, 138]. The damage in abomasum increases the gastrin in the blood. The studies established an association between the elevated blood gastrin levels and depressed feed intake in Ostertagia-infected calves [45]. Reduced appetite is the major factor described in all types of parasitosis in GI-tract. Feed intake is depressed in ruminants by reduced flow of digesta, abnormal gut motility, distension of the reticulo-rumen, abomasum or intestine and raised circulating secretin, gastrin and cholecystokinin (CCK) levels [138].

The nematode H. contortus is blood-sucking parasite and the major clinical sign of this parasite is anaemia. The severity of blood loss is increased additionally by bleeding of raw ulcers created by the worms even after they leave the abomasal wall. The animals lose the iron reserves and haemoglobin level decreases, then their haemopoietic systems become exhausted therefore they can die [161]. Anaemia is also accompanied by hypoproteinemia and oedema which may contribute to death. A common observation in these cases is sub-mandibular oedema termed “bottle-jaw” [143].

Trichostrongylus species parasitize in small intestine, mostly in the proximal part. These worms cause atrophy of the villi concomitant with a hypertrophy and a hyperplasia of the crypts due to an increase in cellular renewal, which contributes to the partial regeneration of the lesions.
Moreover, in the distal part of the intestine, a hypertrophy and a hyperplasia of the crypts associated with elongated villi has been observed during infection with *Trichostrongylus colubriformis*, which evoked an eventual adaptation of the intestine with possible compensatory absorption [67].

*Oesophagostomum* spp., *Chabertia* spp. and *Trichuris* spp. can parasitize in the large intestine. *Oe. columbianum* produce some glandular secretions (cephalic and oesophageal) which are considered responsible for the chronic inflammation in the intestinal wall resulting proliferation of the fibrous tissues. The larvae of this nematode penetrate the mucosa and reach the deeper parts of the sub-mucosa where they encyst and undergo moulting, form gross nodule, which provokes an intestine tissue reaction [105].

Most infections caused by *T. ovis* are light and asymptomatic. In some cases a large numbers of worms cause a diphtheritic inflammation of the caecal mucosa. *T. ovis* penetrates the intestinal wall by their anterior parts. Probably during the process of penetration, they cause mild to moderate degree of damage in the intestinal surface, resulting petechial haemorrhages [105].

The immunity of the host responds to nematode infection with production of specific IgA, IgE, IgG antibodies, eosinophilia and mucosal mastocytosis what is dependent on the activation of T helper (Th) 2 cells [27, 130]. In his study, Perez et al. [112] showed, that T and B lymphocytes and IgG producing plasma cells were recorded in the abomasum and abomasal lymph nodes of goats 3, 7 and 21 weeks post-infection after an experimental infection with *H. contortus*. Marked increase in the secretion of mucus by mucous cells together with an abundant infiltration of eosinophils, mastcells, CD3+ T lymphocytes, CD79a+ B cells, IgG+ plasma cells and globule leukocytes were recorded in the abomasal mucosa, especially at 7 wpi. Except for the globule leukocytes, this reaction decreased substantially by week 21, suggesting this cell type may have been involved in rejection of adult nematodes [112].

### 1.2. Other important gastrointestinal parasites of goats

Other important gastrointestinal parasites are *Eimeria* spp. (Coccidae), *Fasciola hepatica* (Trematoda) and *Moniezia* spp. (Cestoda). Protozoa *Eimeria* species are specific for each animal species and some of them are less or non-pathogenic, even when large numbers of oocysts are present in faeces. In goats are found at least 9 species of *Eimeria*, but most pathogenic species in them are *E. ninakohlyakimovae* and *E. arlongi* [22]. Goats of all ages are susceptible to *Eimeria* spp. infections, but younger animals are more likely to develop the disease. *Eimeria*-infected goat kids show clinical
signs particularly during the weaning period, ranging from non-haemorrhagic to severe haemorrhagic diarrhoea, with accompanying weight loss, dehydration and growth delay [120].

*Fasciola hepatica* cause damages of the liver and bile ducts, which induce digestive disturbances, anaemia, oedema, and developing cachexia and therefore cause economic losses. The highest infection risk is at the end of grazing season, because the snail population increases until autumn.

The tapeworms *Moniezia expansa* and *M. benedeni* generally regarded as of little pathogenic significance but heavy infections may cause unthriftness, diarrhoea and even intestinal obstruction [149]. Heavy infections mostly occur in young animals however in adults the infection generally shows no symptoms.

### 1.3. Anthelmintic treatment

#### 1.3.1. Anthelmintics

Anthelmintic treatment is mostly used as a control implement against GIN infections as it is simple to use and highly effective. Anthelmintics are classified into several groups depending upon their mode of action, range of activity against different parasites or their chemical structure. Based on their range of activity, they are characterized as broad-spectrum or narrow spectrum drugs.

Benzimidazoles are broad-spectrum anthelmintics. Their active substance binds to β-tubulin, a constituent protein present in microtubules, plasma and mitochondrial membranes [113]. This leads to inhibition of microtubule production. Microtubules are usually associated with the information of mitotic spindles during cell division, maintenance of cell shape, cell motility, cellular secretion, nutrient absorption and intracellular transport [83]. Inhibition of microtubules leads to disruption of these functions. They are relatively safe for ruminants, although albendazole has been shown to have teratogenic effects, and should therefore not be used in early gestation [169].

The macrocyclic lactones are the most recently discovered class of anthelmintics and all have broad-spectrum activity and show anthelmintic, insecticidal and acaricidal activities. The macrocyclic lactones all share a base of a 16-member lactone ring, but are split into two groups based on chemical differences, the milbemycins (such as moxidectin) and the avermectins (such as ivermectin and abamectin) [98]. Ivermectin is a member of the avermectin group compounds, which are isolated as fermentation products from actinomycete *Streptomyces avermitilis* [101].
The avermectins/milbemycins bind to glutamate and gamma-aminobutyric acid (GABA)-gated causing a hyper-polarization of nerve or muscle cells, leading to paralysis of movement, paralysis of pharyngeal pump and inhibition of oviposition due to effect on uterus muscles [114]. Therefore, nematodes are unable to move, to feed or to reproduce. Finally, they are quickly removed from the host animal.

Table 1.3.1.1. Overview of some available anthelmintics [33, 38, 62, 88] (Licensed in Lithuania for cattle*, sheep○ and goat□)

<table>
<thead>
<tr>
<th>Anthelmintic class</th>
<th>Active ingredient</th>
<th>Dosage in mg/kg body weight for goats</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I Benzimidazoles</td>
<td>Fenbendazole</td>
<td>7.5-10 mg/kg orally</td>
<td>Panacur*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cerafluke*</td>
</tr>
<tr>
<td>Class II Imidazothiazoles</td>
<td>Levamisole</td>
<td>8-12 mg/kg orally</td>
<td>Levamisole, * ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Administration of injectable product better to be avoided</td>
<td>Levamisol 8%*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dehelmanto *, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Belamisol*, ○</td>
</tr>
<tr>
<td>Class III Ivermectin</td>
<td>0.3-0.4 mg/kg - injectable</td>
<td></td>
<td>Ivomec 1%*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ivermectin PLUS*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bimectin*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Biomectin*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Noromectin*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Promectine*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Levatum Super*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ceveamec*, ○</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intermectin*, ○</td>
</tr>
<tr>
<td>Class III Doramectin</td>
<td>Pour-on usage for goats - no data</td>
<td>Norador*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 mg/kg injectable</td>
<td>Taurador*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dectomax*, ○</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prontax*, ○</td>
</tr>
<tr>
<td>Class III Eprinomectin</td>
<td>0.5-1 mg/kg pour-on</td>
<td>Eprizero*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anamex*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neoprinil POUR-ON*</td>
</tr>
</tbody>
</table>

Levamisole (LEV) belongs to the class of imidazothiazoles and tetrahydropyrimidines, which include pyrantel, morantel and bephenium and is a broad spectrum anthelmintic. Levamisole acts as an antagonist binding to the α-subunit leading to a constant depolarisation of the cells, such as muscle cells, causing spastic paralysis of nematodes [12]. Unfortunately this anthelmintic is not effective against hypobiotic larvae. The study in a sheep flock showed an outbreak of parasitic gastroenteritis in the flock, where the animals were dosed with levamisole at the turnout [123]. Also, levamisole has a narrower therapeutic safety index compared to the benzimidazoles, and should therefore be used with caution in pre-parturition animals [124].
Salicylanilides, organophosphates, piperazine and phenothiazine are narrow-spectrum anthelmintics and not often are used by farmers.

Two new broad-spectrum anthelmintic drug classes were introduced for the control of GIN parasitism in sheep: the amino-acetonitrile derivatives (AADs) [74] and the spiroindoles [121]. However, the usage for goats has not been described.

1.3.2. Anthelmintic treatment regimes

The control of GIN-infections is largely based on strategic or therapeutic use of anthelmintic drugs alone or combined with grazing management [167]. Mostly, these strategic treatments are performed in spring at the beginning of the grazing period to prevent the contamination of the pasture and in autumn at the end of grazing period. On most goat farms the flocks are traditionally treated one time per year in autumn/winter during non-lactating period. This strategic (targeted) treatment is based on the knowledge of the risk, or the parameters that quantify the severity of infection. Anthelmintic treatment was optimised by targeting treatments to groups of animals based on FEC (can be pooled sample), grazing management and/or in relation to parturition in small ruminants [19, 78]. Additional treatments are administered when clinical signs of disease are evident or taking into account the time when animals are moved to other pasture.

Bliss [14] suggested “0-3-6-9” strategic deworming program, which is preventing pasture contamination during the first 60 to 90 days of the grazing season. The repeated deworming with intervals no more than 21 – days prevented from shedding additional worm eggs on the pasture during the first three months of the season, parasite safe grazing can be maintained and parasite burden developing in the animals over the summer grazing season can be significantly reduced concerning the low re-infection. In Lithuania, this strategic treatment was administered to first-season calves at weeks 3, 8 and 13 after turnout with ivermectin and showed significant lower FEC and higher weight gain in the second grazing period [129].

When deworming is combined with grazing management, it is not recommended to practise “drench-and move” regime bearing in mind high risk of development of anthelmintic resistance. This practice allows increasing the population of unaffected (resistant) worms in GI-tract. In temperate areas, the majority of the parasite population (up to 95% of total worm population) is usually found on pasture, and therefore, provides a relatively large reservoir of susceptibility [77]. The source of refugia (susceptible GI nematodes of total population) has to be retained on the
pasture. Molento et al. [102] suggested that animals should be moved prior to drenching and treatment delayed until the desired levels of refugia have built up to the new pasture. The experiment confirmed that lower resistance was in lambs, which were grazed in the similar contaminated pasture compared to those, who were grazed on low-contaminated pasture [165].

Also the practice that the dewormed goats turn out on the pasture at the beginning of grazing season leads to higher development of resistant nematodes [136]. In such situation, pastures are contaminated by overwintered infective larvae and eggs laid by resistant worms.

Even better is, if practicable, to selectively treat only those animals which would ‘most benefit’ from treatment (targeted selective treatment-TST). For the selection to treat the following indicators are used (one or combination):

– FEC,
– weight gain or body condition score,
– milk yield,
– FAMACHA© system.

FEC monitoring is used to help predict the need for treatment against Teladorsagia spp. and Trichostrongylus spp. EPG threshold for the application of TST can be above 300, because this threshold is an arbitrary value derived from studies in Greece, where it was found that apparent clinically healthy dairy sheep and goats often are infected with parasitic burdens shedding over 300 EPG [47, 116]. In the study with lambs of Leathwick et al., [87] the threshold used was 500 EPG mean in the group (predominant species was Haemonchus and Teladorsagia). In another study performed on sheep, TST was based on FEC conducted monthly and only the animals with a GI strongyle egg count above the mean of the group (considered as FEC threshold) were treated [32]. FEC results cannot be generated speedily and make it difficult to apply in practice. That is the need to collect faecal samples from individually marked animals, examine them in the laboratory and then return to the farm again to restrain the flock for a second time, in order to find and treat the heavily infected individuals [116].

The selected treatment based on body condition can be used to determine goats which require anthelmintic treatment. The survey where selection of treatment was based on body weight gain of young animals reduced anthelmintic usage and showed no significant difference in mean body weight compared to intensively suppressive treatment regime (treatment every 4 weeks) [79]. In flocks of adult animals, body condition score (BCS) can be used which is based on an index of 1–5 (score 1 was used for very emaciated animals and the maximum score 5 for very fat animals). All animals in the flocks/herds were examined and those with BCS <2 were
suggested to be treated [47].
As regards the milk production, the studies in dairy goat flocks in France have shown that animals in their first lactation and high milk producing multiparous animals have higher GI strongyle FEC [60]. Targeting anthelmintic treatments using milk production as an indicator reduced anthelmintic treatments by 48–66% [58]. The TST approach was then tested in 11 dairy farms in France in a 2-year survey resulting in the reduction of anthelmintic usage by 40% with no significant associated changes in egg output or milk production observed [57], compared to conventionally treated animals [116].

FAMACHA© system, which is widely used in *Haemonchus* endemic regions, uses the colour of the ocular membranes as an indicator of anaemia to identify individuals at risk of haemonchosis [153]. The categories (Fig. 1.4.1.1) range from 1 – red (nonanaemic) to 5 – white (severely anaemic) [94]. Thereby, as suggested by Van Wyk and Bath [153], only individual animals of the flock showing severe anaemia, i.e. goats scored 3, 4 and 5 and sheep scored 4 and 5, respectively, have to be treated selectively.

These results indicate the suitability of FAMACHA as an additional part of an integrated anthelmintic control of goat flocks in Switzerland [133]. However, there is the limitations of this system in Europe largely concerning the ubiquity of mixed nematode infections and the presence of other blood feeding parasites, for example, liver fluke, such that anaemia alone cannot reliably reflect the impact of roundworms on the animal [18].

![Fig. 1.4.1.1. Official FAMACHA©-anaemia guide](image)
1.4. Anthelmintic resistance

Resistance is the heritable ability of the parasite to tolerate a normally effective dose of the anthelmintic. The parasite is considered resistant if it survives exposure to the standard recommended dose of the anthelmintic and the ability to survive is passed on to its offspring. Survived worms produce eggs giving rise to resistant worms, which re-infect the flock and a further selection for anthelmintic resistance is the result.

The mechanisms of resistance can broadly be divided into two main processes: firstly, into a change in the target molecule and secondly, into a mechanism which inactivates or removes the drug from the environment of the target molecule.

Table 1.4.1. Possible mechanisms of resistance to the major anthelmintic families [168]

<table>
<thead>
<tr>
<th>Anthelmintic family</th>
<th>Mechanism of resistance</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazoles</td>
<td>b-tubulin isotype 1 mutations: F200Y, F167Y</td>
<td>The best studied mutations and probably the most important. F200Y seems to be the most important mutation in H. contortus, but this might not be true for all species.</td>
</tr>
<tr>
<td></td>
<td>b-tubulin isotype 2 mutations: F200Y, F167Y, deletion. Altered metabolism</td>
<td>Also present in H. contortus, field importance unknown. Might be important in triclabendazole-resistant flukes; importance in nematodes unknown, but probably minor.</td>
</tr>
<tr>
<td></td>
<td>Mutations in GluCl and/or GABA-R genes</td>
<td>Molecular evidence from Cooperia oncophora; population genetic evidence from H. contortus.</td>
</tr>
<tr>
<td>Avermectins and milbemycins</td>
<td>Overexpression of P-glycoproteins</td>
<td>Population genetic and some pharmacological evidence. The relative importance of these two mechanisms is yet to be determined.</td>
</tr>
<tr>
<td>Levamisole</td>
<td>Changes in nicotinic acetylcholine receptors</td>
<td>Physiological and pharmacological evidence; no molecular data to date.</td>
</tr>
</tbody>
</table>

Anthelmintic resistance is an increasing problem in helminth control. Different methods, both in vivo and in vitro methods, have been used to detect and monitor [69]. In vitro tests can be divided into two groups based on their effects: pharmacological and biochemical. In the former, the physiological functions of the parasites are directly affected (e.g. production of parasite eggs or larvae), while in the latter, biochemical processes are affected (e.g. drug binding to larval tubulin or eserine to receptors) [159].
1.4.1. Faecal egg count reduction test

The major method for the detection of resistance remains the faecal egg count reduction test (FECRT) that can be used with all anthelmintic groups. Nematode eggs are counted in faeces at the time of treatment and at defined times after treatment; the time depending on the anthelmintic group used. The time after treatment depends on the anthelmintic used: 7 days after LEV, 10-14 after BZ and 14-16 days after a ML. Animals that have not been dosed for 10 weeks, or are known to have had zero egg counts after their last dosing, should be used and a mean FEC of 300 EPG or more is recommended before starting the trial. This test is only reliable if more than 25% of the worms are resistant [29]. The accuracy of the method depends on a correlation between egg counts and worm burdens which is not always present. Nematodes like *Trichostrongylus colubriformis* and *Ostertagia circumcincta* show little correlation whereas *H. contortus* show good correlation [69].

1.4.2. The egg hatch test

The egg hatch assay (EHA) was first described by [86] for the detection of benzimidazoles (BZ)-resistance. After that, this method has been modified and currently, the most commonly used modified version recommended by the WAAVP, is the one according to Coles et al. [29]. It is based on the ovicidal properties of BZ and the ability of eggs of resistant populations to embryonate and hatch in a higher concentration of BZ than can eggs from sensitive populations [159].

1.4.3. The larval development test

The larval development assay (LDA) uses the ability of the anthelmintic to arrest the normal development from eggs to L3 larvae. After incubation the percentage of undeveloped eggs, L1-L2 and L3 larvae is calculated for each drug concentration and for drug-free controls [159]. The sensitivity is generally considered higher than that with the FECRT so AR may be detected when the frequency of resistant alleles within the worm populations is still low. This method can be used to detect BZ, IM and LEV. The test has the great advantage of the simultaneous detection of efficacy/inefficacy of the most used broad-spectrum anthelminthics.

There is a lot of information about GI parasites of small ruminant in European countries, but the data from Lithuania and other Baltic countries
are limited. The prevalence of GI parasites and infection level in goat farms are unknown. There is no data about AR of nematodes in Lithuania. This information is important for observation of AR and control of GI parasites in the European countries.
2. MATERIAL AND METHODS

The work was carried out at the Department of Infectious Disease of the Lithuanian University of Health Sciences within the period 2010–2014. The investigations were performed in compliance with Lithuanian animal welfare regulations (No.B1 – 866, 2012; No. XI – 2271, 2012) and were approved by the Lithuanian Committee of Veterinary Medicine and Zootechnics Sciences (Protocol No. 07/2010).

The study was carried out in three stages (Fig. 2.1):

- **I stage**
  - Epidemiology of GIN infection
  - Efficacy of anthelmintic treatment

- **II stage**
  - Collection of questionnaire data by phone and e-mail

- **III stage**
  - Determination of AR in goat farms

**Fig. 2.1. The design of the study**

2.1. Study farms

2.1.1. Questionnaire survey

The questionnaire survey was undertaken during the year 2012. The total of 37 goat owners from Lithuania was questioned by phone or e-mail. The questions were related to (i) farm management: number of adult animals, breeds, size of pasture, grazing management and (ii) worm control practices: frequency and timing of deworming, usage of anthelmintic products,
determinations of dosage (Accessory 1). Most of surveyed farmers were from Middle- and East-Lithuania.

### 2.1.2. Survey of risk factors for gastrointestinal parasites infection

The study was performed in four dairy breed goat farms, located in different regions of Lithuania during the grazing period (May-November) in 2011 and 2012. The representative farms in terms of flock size and grazing management (Table 1 in Publication I) were selected for the study. On Farms A and B, the grazing period lasted from end of April until the beginning of November. The weaned kids on Farm A grazed in a separate paddock while those on Farm B grazed freely together with adult-tethered goats. The tethered goats grazed by a rotation principle, and returned to the same pastures with 6–8 week intervals. On Farm C, the grazing started in the middle of May for adults, whereas the kids remained indoors with outdoor access to pens for 5 hours/day from the middle of June until the end of August. During this period, the kids were fed a diet comprised of a mixture of hay or fresh cut clover, concentrates, some milk and water *ad libitum*. They also had access to the grass in the pens. Thereafter, the kids were turned out on pasture and grazed together with the adult dairy goats. All goats on Farm C were supplementary fed with clover or lucerne grass/hay and milled grains 2 times/day. The goats on Farm D were permanently stabled all the year around, with 10–15 animals in isolated pens. They were fed a mixture of fresh grass/hay, milled grains, bagasse and vegetables (carrots, beetroot, apples).

Anthelmintics were only used routinely on farms A and C in previous years. Furthermore, toltrazuril (Baycox® Bovis) were used against *Eimeria* infection on Farm A due to the onset of severe clinical signs (diarrhoea and few lethal cases) among the kids.

### 2.1.3. Farms for epidemiological study of gastrointestinal nematodes

The study was started in April 2012 and was performed for one year on two dairy goat farms, located in the eastern part of Lithuania. The grazing period was from the end of April until the end of October on both farms. During housing, the animals were fed with hay/haylage and grains. The kidding period on both farms started at the end of January and lasted until the end of February. The kids on Farm A were weaned after 3 months. Thereafter they were grazed separated from the adults in a paddock with ≈500 kg per hectare (ha) for up to October. The grazing intensity of the adults at Farm A was ≈320 kg/ha. In contrast, the kids on Farm B grazed
freely and in the same paddock as the adult goats (235kg/ha), which were tethered and moved according to a rotation principle and returned to the same position after approximately 6–8 weeks.

Anthelmintics had not been used on any of the two farms for the last 4 months prior to the start of the trial. On Farm A, the yearlings received injectable ivermectin (Ivomec® 1%, 0.3 mg per kg body weight) in early October, whereas the adults were dewormed in late November. In contrast, all goats on Farm B were untreated throughout the study.

2.1.4. Farms for the study of gastrointestinal nematode control in young goats

Forty White Shorthaired breed and 3 months old goats from two commercial farms with high (n=20) and low intensity (n=20) grazing were selected. In the high intensity grazing farm (HIG) the stocking rate was 25 goats/ha, in the low intensity grazing farm (LIG) 4 goats/ha, respectively. All goats were weighed; the faecal and grass samples were collected every second week. After turnout in the end-April the goats grazed together on the permanent paddock. On July 17, the goats in each farm were randomly divided into two equal groups (experimental and control) and were kept on the different plots. The experimental animals were treated with fenbendazole (Panacur® granules) 10mg/kg BW, while the controls were served untreated. Because of higher infection level and contamination of pasture, at the end of the study period (middle of October) all young goats on HIG farm were treated with ivermectin (Ivomec® 1%) 0.3mg/kg BW.

2.1.5. Farms for the anthelmintic resistance study

A total of 9 goat farms, stocking 7–200 animals, in the central and eastern parts of Lithuania, were selected for AR study and visited during August-October 2013. The selected farms reported about the problems with gastrointestinal nematodes and were using regularly anthelmintic treatment once or twice per year. At the sampling day, the animals had not been treated against gastro-intestinal nematodes for at least six weeks.

All farms were tested by egg hatch test (EHT) for the detection of benzimidazole resistance and Micro-agar larval development test (MALDT) for anthelmintic resistance (AR) to benzimidazoles and ivermectin. However, only 5 goat farms were tested for AR to levamisole.
2.2. Parasitological techniques

2.2.1. McMaster method

The faecal samples were taken from the rectum. The number of nematode eggs (EPG) and oocysts per gram of faeces (OPG) were determined by a modified McMaster technique [118] with a sensitivity of 20 eggs per gram of faeces. Thereby, 4 g of faeces was mixed in 56 ml water with tongue blade and left for 30 min.; then the faecal suspension was filtered through cheesecloth into other plastic container. 10 ml of this suspension was pipetted into centrifuge tube and centrifuged for 7 min. at 1200RPM (revolutions per minute). After that, the supernatant was removed with a pipette. Shortly before counting, flotation fluid (zinc chloride solution with density 1.4) was added to the 4 ml mark. The sediment was mixed very carefully, using a Pasteur pipette several times. The both sides of McMaster chamber (2 cell chamber) was filled with the faecal suspension (0.3 ml) and left to rest on the table for 3 min. before counting. The eggs and oocysts were counted in both chamber sides under a microscope at 10 x 10 magnification.

2.2.2. Coprocultures

To determine the genus of gastrointestinal nematodes, the faecal samples were prepared according to Henriksen and Korsholm [51]. Triplicate faecal cultures were prepared for each age group per farm by pooling of 1 g of faeces from the animals within the group and mixed with water and vermiculite. The culture chambers were incubated in the moisture box at 24 °C for 10–14 days. After incubation, the culture chambers were transferred for Baermannisation to the conical glass vessel which was filled with tap water (20–25 °C). The aggregate was left for 24 hours at room temperature and after that, L₃ larvae were collected. From each culture, at least 100 L₃ were morphologically differentiated and identified to the genus or species level [90, 154].

2.2.3. Collection of larvae from grass

The examination of pasture contamination with L₃ larvae was performed according to Fernandez et al. [44] method. Three replicate herbage samples of approximately 400 g of weight were collected from the pastures grazed by goats for the determination of the numbers of nematode larvae. Each herbage sample was collected while walking across the pasture in a W-
shaped pattern and retrieving 3 subsamples at every 20 steps. Grass within 20 cm of faecal pellets was avoided. The collected grass sample was placed in the water in a bucket. Next day, the grass was washed above a sieve (aperture 38 microns) and the larvae were collected. The sediment (with larvae) was transferred into glass and this suspension prepared for Baermannisation. The grass sample was dried for 2–3 weeks. The larvae were counted and the results were expressed as the number of L₃ per kg of dried grass.

2.2.4. Baermann technique

The Baermann method was used for recovery of the L₁ larvae of lung nematode from the faeces and L₃ larvae of gastrointestinal nematode from coprocultures and grass samples. This technique is based on the active migration or movement of larvae. Fresh faeces were used for this method. Using a spatula 5–10 grams of faecal material were weighed and was laid on cheesecloth. The four corners of the cheesecloth were taken together and closed with a clip. This pouch was clipped with the stick and placed in the conical glass with lukewarm water. These pouches were left to stand at room temperature for 24 hours. After an overnight incubation, the larvae migrated out into the water where they sank to the bottom of glass. Approximately 5 ml of fluid was collected with a pipette into test tube. The tubes were left to sediment for 24 hours. By using Pasteur pipette, 3 drops of the sediment fluid were transferred on microscope slide. The larvae were fixed with Lugol’s iodide. They were identified according Van Wyk et al. [154] recommendations.

2.2.5. Sedimentation method

The sedimentation method was used to detect *Fasciola* sp. eggs in the faeces. 10 grams of faeces were weighed using a spatula and placed into container 1. 250 ml of tap water were poured on the faeces and mixed (stirred) thoroughly with a tongue blade. The faecal suspension was filtered through a teastrainer into a conical glass. After 30 min., the supernatant was removed and 250 ml of tap water were repeatedly poured on the sediment. After 30 min., the supernatant was removed and 50 ml of tap water were poured on the sediment. After 5 min., the supernatant was removed and one drop of methylene blue added. The sediment was transferred into Petri dish and examined by microscope at 10 x 4 magnification.
2.2.6. Post-mortem examination

For species of nematodes and inhibited L₄ larvae determination the viscera of young goats were collected. The abomasum, small and large intestine were separated and worm burdens were collected according Grønvold [50]. The contents were washed separately into bucket and adjusted to 10 litres by tap water. Approximately 10% of subsamples from the contents of the abomasum were washed separately through mesh sieves with an aperture 38 µm, the small intestines – 53 µm and large intestines 500 µm. In addition, the abomasal mucosa was removed by scraping with a knife. These scrapings were digested in a solution of 1N HCl acid dissolved by 8 g of pepsin (1:3000) per litre for half an hour at 39°C to retrieve arrested larvae. After that, the mucosal suspension was sieved through the sieve with aperture of 38 µm. All retained parasites were preserved with Lugol’s iodide (10%) until identification and enumeration. Nematodes from each compartment were collected and counted, and the first 50 adult nematodes and L₄ larvae were identified [9].

2.2.7. Recovery of worm eggs from faeces

Faecal samples were taken rectally or from the ground (fresh) from 7–15 goats older than 6 month of age. About 6–10 grams were taken from each sample and placed in one plastic bag (pooled sample; totally 100–150 grams) and mixed. Later the samples were prepared for anaerobic conditions. Approximately 1/3 of pooled sample were put into plastic/glass container (250 ml). The container was filled with water up to 2/3 of its volume, closed and mixed well. After this, the container was filled to the top and closed tightly. The anaerobically stored samples were examined in five days.

In the laboratory, the nematode eggs were collected by sieving the faeces through three stacked sieves with apertures of 250 µm, 100 µm and 20 µm, respectively. The material collected on the 20 µm sieve was washed with water into glass and left for sedimentation. After 15–20 min. the supernatant was removed and the sediment transferred into tubes with cover. The tubes were centrifuged for 2 min at 1500 RPM. The supernatant was removed again and the saturated sodium chloride flotation was poured on the sediment.

The tubes were mixed, filled until formation of meniscus, plugged and re-centrifuged for 2 min. 1500 RPM. After centrifugation, the material (with eggs) from the corks was washed into a glass. The water-egg suspension was washed with deionised water into other glass. Finally, eggs
concentration in 10 µl of water-egg suspension was calculated. The water was removed or diluted to 100–150 eggs/100 µl in egg suspension.

2.2.8. Egg hatch test

Egg hatch test was used to estimate AR to BZ as the method described by Coles et al. [29]. A stock solution of thiacendazole (TBZ) (Sigma-Aldrich, Germany) was prepared by dissolving the pure compound in dimethyl sulfoxide (DMSO). The final concentration was prepared by adding 10µl of the TBZ solution to 1.99 ml of an aqueous suspension of approximately 150 eggs/ml. The final TBZ concentrations were 0.05, 0.1, 0.3, 0.5 and 1.0. 0.5% DMSO without anthelmintic was also included in the test as a control. The dosed egg suspensions were incubated in 24-well plates (Nuncleone, Denmark). After incubation for 48h at 27˚C, the test was stopped by adding 10–25 µl of Lugol's iodine to each well and all eggs and larvae were counted. The test was performed with two replicates.

2.2.9. Micro-agar larval development test

We have used the MALDT to detect AR to BZ, IVM and LEV described by Coles et al. [29]. The test was performed on 96-well microtitre plates. Stock drug solutions of thiabendazole were serially diluted 1:2 with dimethyl sulphoxide (DMSO) to produce 12 final concentrations ranging from 0.0006 to 1.28 µg/ml and ivermectin aglycone (IVMA) (Sigma-Aldrich, Germany) from 0.084 to 173.6 µg/ml, respectively. Stock drug solutions of levamisol hydrochloride (Sigma-Aldrich, Germany) were serially diluted 1:2 with deionised water to produce the final concentrations ranging from 0.0156 to 32 µg/ml.

Subsequently, 12 µl of the drug solutions were mixed with 150 µl of 2% Bacto agar at 45 °C. After solidification of the agar, 10 µl of eggs in a 0.3 mg/ml solution of amphotericin B (final number of eggs per well was 30–50) were mixed with 10 µl of yeast extract and then added to the agar. For control wells, no drug and only DMSO (1.6%) in TBZ and IVMA test plates and deionised water in LVM test plates were used. The yeast extract was prepared as described by Hubert and Kerboeuf [66] (e.g. 1 g of yeast extract in 90 ml of 0.85% NaCl was autoclaved for 20 min, and then 27 ml of this solution were mixed with 3 ml of 10× concentrated Earle's solution). The plates were incubated for 7 days at 25°C. Larvae were then killed with 50 µl Lugol's iodine solution, and all eggs and first-/second- and third-stage larvae in each well were counted under a microscope before transferring the suspension on the glass.
2.3. Other analysis

2.3.1. Weather conditions

The data on monthly precipitation and average temperature were obtained from the meteorological stations situated 7–11 km from the examined farms. For comparison, the data covering a 30-year period (1961–1990) were obtained from the Lithuanian Hydrometeorological Service.

2.3.2. Clinical observations

On each sampling (2 weeks intervals) the clinical exposure of goats was observed: body condition and consistency of faeces. Faecal consistency was documented and graded into 3 categories (from 1 to 3): solid, pasty and liquid diarrhoea. On treatment study the young goats were weighed on each sampling day.

2.3.3. Statistical analysis

For descriptive statistics from questionnaires, the means of EPG/OPG, the larva of coprocultures were calculated using BMI SPSS Statistics (Version 21). The prevalence of nematode infections in the GI tract and the standard 95% confidence intervals (CI) were also calculated. Worm burdens on the two farms were compared using one-way ANOVA. Statistical comparison of quantitative faecal egg/oocyst counts between farms and sampling periods (months, every two weeks) was performed using Repeated Measures Analysis of Variance (ANOVA).

The Multinomial Logistic Regression was used to determine the dependence on animal species, grazing management and treatment control practices, selection of treatment date and determination of dosage. The Kruskal-Wallis ANOVA was used for calculating the significant differences regarding flock and pasture size, grazing intensity and number of anthelmintic treatments in different animal species farms.

The effect of categorical variables as age (kids – under 6 months, young animals – 6–12 months and adults – over 12 months) and grazing management (open-grazing, tethered, set-stocked of young, young grazing with adults, feed supplementation (with two anthelmintic treatments) and zero-grazing) was assessed for potential association with strongyle eggs and Eimeria spp. counts in faeces using Linear Mixed Models procedure. The first order autoregressive (AR1) correlation structure was used by lower
Akaike's Information Criterion (AIC).

The effect of various factors on *Strongyloides* spp. and *Trichuris* spp. infections were also assessed. The data contained repeated binary measures of infection in goats with *Strongyloides* spp. and *Trichuris* spp. as dependant variables along with a fixed recording of age (young and adult), grazing management (open-grazing, tethered, set-stocked of young, young grazing with adults, feed supplementation (with two anthelmintic treatments) and zero-grazing), season (spring, summer and autumn) and treatment or no treatment with anthelmintics before/during the study. The Generalized Estimating Equations (GEE) were used to fit a repeated measures logistic regression with these data, using the main effect model with unstructured correlation structure. The Independence Model Criterion (QIC) was used to choose between two correlation structures, given a set of model terms. The first-order autoregressive (AR1) structure was obtained accordingly as lower to this criterion (QIC).

The data were analysed by a logistic regression model to determine LC50 and using Microsoft Excel software (Version 2010). The degree of AR was expressed as a resistance factor (RF) and was calculated as the values of LC50 and LC99 of the resistant isolates divided by the respective values of the susceptible isolates.

The value P<0.05 was considered statistically significant.
3. RESULTS

3.1. Prevalence of parasite species and epidemiological survey of goat farms in Lithuania

To determine the internal parasite species in goats in Lithuania the faecal samples from nine goat flocks were examined. For the study of the seasonal infection patterns, the two farms were visited and sampled on the regular basis and the remaining seven farms were visited and sampled once.

3.1.1. Prevalence of parasite species in goat farms

Faecal egg count. The study in 9 goat farms showed that overall prevalence of digestive nematode egg excretion was 98.9% and 93.4% in adult and young goats respectively (Table 3.1.1.1). In all farms *Strongyloides papillosus* was detected and the infection level ranged from 15.4% to 70.6% of goats. *Trichuris* spp. infection was more common in adult goats (P<0.05). *Capillaria* spp. species were not found in young goats and were determined in 4.3% of adult goats.

Table 3.1.1.1. Faecal egg count in adult and young goats

<table>
<thead>
<tr>
<th>Parasites present</th>
<th>Prevalence, %</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>Young</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
</tr>
<tr>
<td>Trichostrongyliids</td>
<td>96.8</td>
<td>969±1213</td>
</tr>
<tr>
<td>S. papillosus</td>
<td>33.1</td>
<td>52±160</td>
</tr>
<tr>
<td>Trichuris</td>
<td>22.7</td>
<td>18±38</td>
</tr>
<tr>
<td>Capillaria</td>
<td>2.6</td>
<td>1±5</td>
</tr>
</tbody>
</table>

Coprocultures. The following genera of nematodes were identified: *Teladorsagia* spp. (42%), *Trichostrongylus* spp. (23%), *Oesophagostomum* spp. (11%), *Chabertia* spp. (8%) and *H. contortus* (17%). Larvae of *Teladorsagia* spp. were the most prevalent larval type in the coprocultures from goats in both age groups; however *H. contortus* larvae (81-89%) dominated on one goat farm (Fig.3.1.1.1). *Trichostrongylus* spp. and *H. contortus* were more frequent in fresh faeces, but the difference was not statistically significant (Fig.3.1.1.2).
Fig. 3.1.1.1. Prevalence of third stage nematode larvae in goat farms

Fig. 3.1.1.2. Percentage of $L_3$ larvae in adult and young goats
3.1.2. Prevalence of other important parasites in goats

Prevalence of *Eimeria* spp. was 100% in adult and young goats on 9 farms. The eggs of tape-worm *Moniezia* spp. were determined in 2.2% of adult goats and in 4.9% of young goats and this parasite was found in 4 farms of 9 (Accessory 3). Lung worm survey showed high prevalence of *Muellerius capillaris* in goats (Table 3.1.2.1), where infection was 80.8% and 85.2% in adult and young goats, respectively. *Fasciola hepatica* was present in one goat farm (8.6%).

Table 3.1.2.1. Prevalence and the mean of *L*. larvae of lung worms in goats

<table>
<thead>
<tr>
<th>Farm Nr.</th>
<th>N positive/tested</th>
<th>Prevalence, %</th>
<th><em>Muellerius capillaris</em> (larvae/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adult</td>
<td>Young</td>
</tr>
<tr>
<td>1.</td>
<td>5/13</td>
<td>38.5</td>
<td>13.8</td>
</tr>
<tr>
<td>2.</td>
<td>18/20</td>
<td>90.0</td>
<td>44.0</td>
</tr>
<tr>
<td>3.</td>
<td>9/11</td>
<td>81.8</td>
<td>4.9</td>
</tr>
<tr>
<td>4.</td>
<td>16/16</td>
<td>100</td>
<td>131.6</td>
</tr>
<tr>
<td>5.</td>
<td>10/12</td>
<td>83.3</td>
<td>58.2</td>
</tr>
<tr>
<td>6.</td>
<td>14/20</td>
<td>70</td>
<td>1.4</td>
</tr>
<tr>
<td>7.</td>
<td>20/20</td>
<td>100</td>
<td>88.4</td>
</tr>
<tr>
<td>8.</td>
<td>10/12</td>
<td>83.3</td>
<td>73.1</td>
</tr>
<tr>
<td>9.</td>
<td>10/12</td>
<td>83.3</td>
<td>23.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>82.4</td>
<td>49.9</td>
</tr>
</tbody>
</table>

3.1.3. Epidemiology of gastrointestinal nematode infection in goats on two farms

*Parasitological examination.* In the beginning of the study (April), the shedding of strongyle eggs from the adult goats was below 500 EPG (Fig. 2A in Publication II). From May FEC gradually increased on Farm A and in July on Farm B. Peak values of 1250 and 780 EPG were observed in September on each farm, respectively. On Farm A, where all goats were dewormed in October, the FEC were significantly (P<0.005) reduced during housing period compared to Farm B.

In April, the FEC in the young goats were ≤20 EPG on both farms (Fig. 2B in Publication II). On Farm A, the FEC increased from September and remained high (≈4000 EPG) until deworming in October. At this peak, anaemia and diarrhoea was also observed in 15% of the young goats on Farm A. On Farm B, the FEC gradually increased from October but was significantly lower compared to Farm A (P<0.001). During housing
(November-April), FEC in the goats gradually increased and there was a marked rise in the end of April 2013 on both farms. In this spring, the rise of FEC manifested as diarrhoea, anaemia and submandibular oedema in 6% of young goats.

Faecal cultures showed that strongyle nematode population in adult and young goats consisted of Teladorsagia (42%), Trichostrongylus (26%), Oesophagostomum (13%), Chabertia (11%) and Haemonchus (8%). The dominant genera in the adult goats on both farms were Teladorsagia and Trichostrongylus. Haemonchus, which is the most pathogenic genus in goats, was only observed during the grazing period (May-October) both on Farm A (3–35%) and on Farm B (8–31%). After deworming in November, Haemonchus larvae were not observed in faecal cultures on Farm A until March 2013, when 2–3% of goats were found positive. The proportion of this parasite in faeces of goats on untreated Farm B was 1–4% during housing period, however in April 2013 the infection level increased to 38% of investigated animals.

The young goats started to shed nematode eggs in May. However, Haemonchus was not detected until June. However, afterwards the infection level started to increase. In August–October on Farm A, 16-21% of young goats were positive to Haemonchus. This was significantly different from Farm B, where 4–14% of the young goats shed Haemonchus (P<0.05). The deworming of young goats on Farm A significantly reduced the level of Haemonchus eggs in faeces compared to untreated Farm B (P<0.05).

Pasture contamination. The total number of L3 larvae at the start of the study was low and decreased until late June (Fig. 1 in Publication II). At the end of June, contamination with L3 increased and the peak of contamination was observed at the end of July in the paddock used by adult goats on Farm A, and the common pasture on Farm B. Two weeks later in August, the pasture contamination peaked on the pasture grazed by the young goats on Farm A (2216 L3/kg dry matter). A second wave of L3 was observed in late October. Teladorsagia was dominant also in the pasture samples (42–100%). Haemonchus was detected for the first time in mid-May and afterwards during the August–October the pasture contamination increased to 9–50%.

Worm burden. Eleven species of GI nematodes were identified, in the abomasum: Te. circumcincta – 52% (95% CI: 42–62%), Te. trifurcata – 34% (95% CI: 23–47%), T. axei – 7% (95% CI: 3–12%), H. contortus – 7% (95% CI: 1–12%), in the small intestine: T. capricola – 45% (95% CI: 34–56%), T. colubriformis – 43% (95% CI: 32–52%), T. vitrinus – 11% (95% CI: 7–16%), Strongyloides papillosus – 1% (95% CI: 0–2%), and in the large intestine: Oe. venulosum – 65% (95% CI: 52–80%), Chabertia ovina –
34% (95% CI: 19–49%), *Trichuris ovis* – 1% (95% CI: 0–3%).

Other analyses. The monthly temperatures were comparable on both farms (Fig. 3.1.2.1). The highest average temperature (+19.6 °C) was observed in July. In comparison to long-term (1961–1990) average values, the temperature was on 1–3 °C higher during the grazing season on both farms. However, winter and early spring temperatures were lower than the long-term average, except in February. The average precipitation was higher compared to long-term average during grazing on Farm A, except September (Fig. 3.1.2.2). On Farm B, the highest rainfall was recorded in April (74.6 mm) and in July (138.6 mm).

Fig. 3.1.2.1. Average monthly temperature in the study areas compared with the average figures for period 1961–1990

Fig. 3.1.2.2. Average monthly rainfall in the study areas compared with the average figures for period 1961–1990
3.2. Analysis of risk factors for gastrointestinal nematode infection

**Parasitological examination.** In the beginning of the study, the number of trichostrongylid eggs in adult goat faeces was low in both study years (Fig. 1a in Publication I). Furthermore, it started to increase reaching the peak values in July/August (Farm B), September (Farm A) and October (Farm C). The highest egg excretion was recorded on Farm B (P<0.001) during the first year and on Farm A (P<0.001) during the second year of the study. The lowest egg excretion was recorded on Farm D (P<0.05) where 43.8% and 60.0% of goats were not infected with trichostrongylids in 2011 and 2012, respectively.

In the beginning of the study, the egg excretion in young goats was low on Farms A and B (Fig. 1b in Publication I). Furthermore, it started to increase reaching the peak values in September (Farm B) and September/October (Farm A). The highest excretion of eggs was observed in young goats on Farm B (P<0.05) in 2011, and on Farm A (P<0.001) in 2012. Egg excretion in young goats started in June and remained low throughout the study on Farms C and D. On these farms, the young goats have shown the lowest infection level with trichostrongylids (41.4 and 82.9%, respectively).

Analyses of nematode larvae from faecal cultures showed that *Teladorsagia* was the dominant genus on all farms. *Haemonchus contortus* was recorded only on Farms A and B, while it was not registered on Farms C and D (Publication I).

The number of larvae was low (51–464 L₃/kg) after turn-out of animals during the study in all investigated farms (Fig. 2 in Publication I). In June–July, 2011 the numbers of larvae increased gradually reaching the values of 2210 (P<0.05) and 1730 (P<0.05) L₃/kg of dry grass on Farms B and C. The larval contamination on Farm A increased in July on the pasture grazed by the adults and one month later on the pasture for the young stock. However, the amount of larvae was lower compared to those on Farms B and C (P>0.05).

In the second year of the study, contamination of grass with infective L₃ stage larvae demonstrated a different pattern. The numbers of larvae were low throughout the season on Farms B and C while it increased in August (P>0.05) on the pasture grazed by the young animals on Farm A.

**Risk factors.** The results from the Linear Mixed Model analysis showed higher (P<0.001) trichostrongylid egg counts in young (6–12 months) goats and kids when compared to those of adult animals (Table 2 in Publication I). The output of trichostrongylid eggs was significantly reduced with zero-grazing management, when compared to the one in other systems.
The impact of significant risk factors on the presence of *Strongyloides* and *Trichuris* was evaluated (Table 3 in Publication I). The GEE model suggested that young goats were significantly less infected with *Strongyloides* and *Trichuris* in the summer period. The open-grazing and tethered grazing management has decreased the incidence of *Strongyloides* infection when compared to those of zero-grazing. The presence of regular anthelmintic treatments on farms was a significant regressor of *Strongyloides* infection.

### 3.3. Efficacy of treatment on two different grazing intensity farms

At start of grazing, the young goats were not infected with gastrointestinal nematodes. Until the end of July, EPG were low (<400) in both farms (Figs. 3.3.1.1 and 3.3.1.2). On week 12 of grazing (on 17 of July), the experimental animals were treated and EPG significantly reduced (P<0.05) compared to controls. However, in the control group of HIG farm EPG significantly increased after 2 weeks, and the highest increment was registered in September/October. In late August, the control goats of HIG farm showed a clinical signs of parasitic infection. The infection level of control goats in LIG farm increased gradually and the peak of EPG was registered in October.

The larval-culture examination showed that the most prevalent nematodes in young goats were *Teladorsagia* spp. and *Trichostrongylus* spp. Furthermore, *H. contortus* larvae were detected in June and the highest infection level was registered in September.

![Faecal egg count in HIG farm. Arrow indicates the time of treatment of experimental young goat group](image)

*Fig. 3.3.1.1.* Faecal egg count in HIG farm. Arrow indicates the time of treatment of experimental young goat group
Fig. 3.3.1.2. Faecal egg count in LIG farm. Arrow indicates the time of treatment of experimental young goat group

In both farms, the herbage larval count was low (<300 l/kg dry herbage) until the beginning of July (Figs.3.3.1.3 and 3.3.1.4). Afterwards, the number of larvae increased and the peak was registered in the beginning of August. After the treatment, the pasture contamination in experimental plot was lower compared to controls, but the difference was not statistically significant.

Fig. 3.3.1.3. Number of $L_3$ larvae on herbage of HIG farm. Arrow indicates the treatment of experimental young goat group
Fig. 3.3.1.4. Number of $L_3$ larvae on herbage of LIG farm. Arrow indicates the treatment of experimental young goat group

The body weight of young goats was 9–11 kg on both farms at the start of the experiment. During the study, the young goats of LIG farm added more weight compared to HIG farm and on the treatment day the weight was 15–18 kg and 18–21 kg on HIG and LIG farms. At the end of investigation, the weight of the treated young goats was 17–25 kg on HIG and 23–30 kg on LIG, respectively. The body weight of the experimental goats was higher by 2.9 kg and by 2.4 kg on HIG farm and LIG farm respectively compared to controls (P<0.05). The examination of faecal consistency showed the changes from late August, when 2 goats of 10 in the control group of HIG farm excreted pasty faeces. After 2 weeks, these goats had diarrhea until the end of the study and 6 goats excreted pasty faeces. In the treated group, only one goat was with pasty faeces in October. However during the study in LIG farm, faecal consistency changes were not observed.

3.4. Questionnaire survey on the gastrointestinal nematode control practices used on Lithuanian goat farms

A survey with questionnaires showed that 100% of goats were for milk production; however the young goats until 8 moths often were slaughtered for meat. Most prevalent goat breeds in Lithuania were: White Shorthaired, Saanen and their crossbreeds; infrequently presented were Nubian and Alpine goats.
Table 3.4.1. General management characteristics of Lithuanian goat farms

<table>
<thead>
<tr>
<th></th>
<th>Goat farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farms (N)</td>
<td>37</td>
</tr>
<tr>
<td>Number of adult animals (mean±SD)</td>
<td>16.8±38.2</td>
</tr>
<tr>
<td>Pastures ha (mean±SD)</td>
<td>4.4±9.5</td>
</tr>
<tr>
<td>Animals/ha (mean±SD)</td>
<td>6.3±3.7</td>
</tr>
<tr>
<td>Grazing management (N (%))</td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Tethered</td>
<td>23 (62.2%)</td>
</tr>
<tr>
<td>Permanent pasture</td>
<td>5 (13.5%)</td>
</tr>
<tr>
<td>Rotated pasture</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>Zero-grazing</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>Other animals on pasture (N (%))</td>
<td></td>
</tr>
<tr>
<td>Only goats</td>
<td>30 (81.1%)</td>
</tr>
<tr>
<td>Sheep</td>
<td>6 (16.2%)</td>
</tr>
<tr>
<td>Cattle</td>
<td>1 (2.7%)</td>
</tr>
</tbody>
</table>

The survey of main characteristics on farms (Table 3.4.1.) showed that the majority of goats were grazing tethered on the pasture (62.2%) and grazing intensity was 6.3 animals/ha. On three farms, goats grazed mixed with sheep and cattle.

Table 3.4.2. Worm control practices on goat farms

<table>
<thead>
<tr>
<th>Worm control factor</th>
<th>Number of goat farms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of treatments</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>17 (46.0)</td>
</tr>
<tr>
<td>1 per year</td>
<td>15 (40.5)</td>
</tr>
<tr>
<td>2 per year</td>
<td>5 (13.5)</td>
</tr>
<tr>
<td>3 per year</td>
<td>0</td>
</tr>
<tr>
<td>Determination of treatment time</td>
<td></td>
</tr>
<tr>
<td>At housing</td>
<td>12 (60.0)</td>
</tr>
<tr>
<td>At housing and in spring</td>
<td>5 (25.0)</td>
</tr>
<tr>
<td>Combination</td>
<td>0</td>
</tr>
<tr>
<td>Clinical sings/scouring</td>
<td>3 (15.0)</td>
</tr>
<tr>
<td>Calculation or estimation of weight</td>
<td></td>
</tr>
<tr>
<td>Weighing (heaviest)</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>Visual appraisal</td>
<td>16 (80.0)</td>
</tr>
<tr>
<td>By veterinarian</td>
<td>2 (10.0)</td>
</tr>
</tbody>
</table>

Chemotherapy and/or chemoprophylaxis in goats were used by 54% of farmers and the mean of treatment frequency in goat farms was 1.2 times/year. The majority of goat farms used a single treatment per year and 60% of animals were treated after the start of housing period between November–December.
The farmers preferred to determine the weight of goats by visual appraisal – 80% of respondents. 10% of farmers chose the doses based on animal weighing whereas other 10% invited veterinary surgeons whose method of determining the doses is unknown.

**Table 3.4.3. Usage of anthelmintic classes on goat farms**

<table>
<thead>
<tr>
<th>Anthelmintics</th>
<th>Number of goat farms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazoles (ALB,FBZ)</td>
<td>4 (20.0%)</td>
</tr>
<tr>
<td>Imidazothiazole (LEV)</td>
<td>3 (15.0%)</td>
</tr>
<tr>
<td>Macrocyclic lactones (IVM)</td>
<td>13 (65.0%)</td>
</tr>
<tr>
<td>Rotation of drug class</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (15.0%)</td>
</tr>
<tr>
<td>No</td>
<td>17 (85.0%)</td>
</tr>
</tbody>
</table>

The most commonly used classes of anthelmintics were macrocyclic lactones as ivermectin (Table 3.4.3). Benzimidazoles (albendazole or fenbendazole) and levamisole for goats’ treatment were used only randomly. In addition, 85% of respondents used the same anthelmintics for several years.

3.5. A survey of anthelmintic resistant nematode parasites in Lithuanian goat farms

The AR survey on 9 goat farms was conducted using EHT and MALDT. These two tests were used to detect AR to benzimidazoles. The nematode population in farm was considered resistant if lethal dose was (LD) 50>0.1µg/ml and LD 99>0.3 µg/ml in EHT. This test showed (Table 3.5.1) that 5 of 7 farms (71%) had low level of nematodes resistance to benzimidazoles (near resistant). The AR level of nematode population on farms was <10%.

The farms were classified as resistant by MALDT, when L₃ larvae were found in concentration 0.04 µg/ml for thiabendazole, 21.6 µg/ml for IVM aglycone and 2 µg/ml for levamisole [34]. These are minimal inhibitory concentrations (MIC) in which development of larvae to the L₃ stage after the incubation period are observed [157]. The calculated values LD50 or LD99 were used as additional information. The results of the MALDT showed AR to benzimidazoles in two farms: in one farm the resistance was low (<10%) and in the second it was moderate (10–25%) (Table 3.5.1)
Table 3.5.1. Resistance to benznimidazoles by EHT and MALDT on goat farms. The population was resistant, if LD 50>0.1µg/ml and LD 99>0.3 µg/ml in EHT, while in MALDT - MIC: 0.04 µg/ml

<table>
<thead>
<tr>
<th>Goat farm</th>
<th>EHT (µg/ml)</th>
<th>LDT TBZ (µg/ml)</th>
<th>Resistance status</th>
<th>Resistance status (-/+ L3 in MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD 50</td>
<td>LD 99</td>
<td>LD 50</td>
<td>LD 99</td>
</tr>
<tr>
<td>1.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>-</td>
<td>0.0098</td>
</tr>
<tr>
<td>2.</td>
<td>0.0655</td>
<td>0.32</td>
<td>SN</td>
<td>0.065</td>
</tr>
<tr>
<td>3.</td>
<td>0.1085</td>
<td>0.2337</td>
<td>S</td>
<td>0.0089</td>
</tr>
<tr>
<td>4.</td>
<td>0.0769</td>
<td>0.3375</td>
<td>SN</td>
<td>0.0091</td>
</tr>
<tr>
<td>5.</td>
<td>0.0524</td>
<td>0.3108</td>
<td>SN</td>
<td>0.0055</td>
</tr>
<tr>
<td>6.</td>
<td>0.0985</td>
<td>0.2484</td>
<td>S</td>
<td>0.0087</td>
</tr>
<tr>
<td>7.</td>
<td>0.0577</td>
<td>0.8102</td>
<td>SN</td>
<td>0.0102</td>
</tr>
<tr>
<td>8.</td>
<td>0.0595</td>
<td>0.5648</td>
<td>SN</td>
<td>0.0074</td>
</tr>
<tr>
<td>9.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>-</td>
<td>0.0091</td>
</tr>
</tbody>
</table>

N.D. – no data

Table 3.5.2. Resistance to ivermectin on goat farms. AR status based on MIC: 21.6 ng/ml

<table>
<thead>
<tr>
<th>Goat farm</th>
<th>IVMA. (ng/ml)</th>
<th>Resistance status (-/+ L3 in MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD 50</td>
<td>LD 99</td>
</tr>
<tr>
<td>1.</td>
<td>5.4917</td>
<td>531.2146</td>
</tr>
<tr>
<td>2.</td>
<td>19.2266</td>
<td>51.4288</td>
</tr>
<tr>
<td>3.</td>
<td>34.0979</td>
<td>890.8583</td>
</tr>
<tr>
<td>4.</td>
<td>20.5244</td>
<td>2075.6155</td>
</tr>
<tr>
<td>5.</td>
<td>29.048</td>
<td>5917.0078</td>
</tr>
<tr>
<td>6.</td>
<td>13.8182</td>
<td>4298.6019</td>
</tr>
<tr>
<td>7.</td>
<td>19.1107</td>
<td>1241.9631</td>
</tr>
<tr>
<td>8.</td>
<td>9.3511</td>
<td>31203.57</td>
</tr>
<tr>
<td>9.</td>
<td>1.8309</td>
<td>311.8179</td>
</tr>
</tbody>
</table>

The MALDT showed resistance to ivermectin on all 9 goat farms (Table 3.5.2). Moderate resistance (10–25%) was detected in 3 (33.3%) and high resistance (>25%) in 6 farms (66.7%).

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**Table 3.5.3.** Resistance to levamisole on goat farms. AR status based on MIC:2.0 µg/ml

<table>
<thead>
<tr>
<th>Goat farm</th>
<th>LD 50 (µg/ml)</th>
<th>LD 99 (µg/ml)</th>
<th>Resistance status (/-+ L₃ in MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.2584</td>
<td>0.904</td>
<td>S (-2.0)</td>
</tr>
<tr>
<td>2.</td>
<td>0.1964</td>
<td>2.7332</td>
<td>S (-2.0)</td>
</tr>
<tr>
<td>3.</td>
<td>0.1673</td>
<td>1.2733</td>
<td>S (-2.0)</td>
</tr>
<tr>
<td>4.</td>
<td>0.2692</td>
<td>1.067</td>
<td>R (+2.0)</td>
</tr>
<tr>
<td>5.</td>
<td>0.1609</td>
<td>1.3994</td>
<td>S (-2.0)</td>
</tr>
<tr>
<td>6.</td>
<td>0.082</td>
<td>0.7832</td>
<td>S (-2.0)</td>
</tr>
<tr>
<td>7.</td>
<td>0.2242</td>
<td>1.69</td>
<td>S (-2.0)</td>
</tr>
<tr>
<td>8.</td>
<td><strong>0.0193</strong></td>
<td><strong>1.36</strong></td>
<td>R (+2.0)</td>
</tr>
<tr>
<td>9.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>-</td>
</tr>
</tbody>
</table>

N.D. – no data

No resistance to levamisole was found on 6 farms (75%) (Table 3.5.3). Resistance to levamisole with low percentages (<10%) of developed L₃ larvae was found on 2 farms (25%).
4. DISCUSSION

4.1. Seasonal dynamics of gastrointestinal nematode infection and the benefit of treatment in goat farms

The seasonal dynamics of nematode infection are the consequence of complex inter-relationships between the goats, their husbandry and the prevailing climate [163]. Lithuania is localised in temperate climate region where the climate is contrasted between summer and winter. Cold winter stops the life cycle of the majority of gastrointestinal nematodes on pasture and helminths have to be adopted to survive during this period.

Our study is the first survey on the helminth fauna in goats throughout Lithuania. This prevalence survey on GIN infections in goats has confirmed that almost every goat grazing on pastures is infected with trichostrongylids. The results from the performed study showed that adult goats excreted from 221.3 to 3012.5 EPG and young goats from 288.6 to 2260 EPG, respectively (Accessory 2). Our findings that goats in our climate zone have high infection level with GIN are in concert with the results described by Scheuerle [131].

In our study of seasonal dynamics, the young goats, as expected, were uninfected at the start of grazing, whereas adult goats excreted ≈500 EPG. It has been observed earlier that FEC levels in adult goats increased from May and with a gradually larger proportion of *H. contortus*, indicating the existence of a spring peri-parturient rise due to depressed immunity against trichostrongylids during lactation periods [21, 26]. It can be assumed that adult animals served as the source for pasture contamination with trichostrongylid eggs in spring and early summer leading to increased numbers of infective L₃ stage larvae on pastures in July and August. In adult animals, the excretion of trichostrongylid eggs markedly increased in August and September, while in young goats in September or October as a result of increased contamination of pastures in July and September.

Generally, goats get infected with mixed nematodes comprising species of the 5 principal genera as: *Teladorsagia* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Chabertia* spp. and *H. contortus*. However, *Teladorsagia* was the most prevalent genus observed in this study irrespective of goat age and farm management. This genus was similarly predominant on goat farms in Norway [37], France [24], Greece [110] and Spain [150]. Under colder laboratory conditions, *Teladorsagia cieumcincta* reach the infective stage to a larger extent than *Trichostrongylus colubriformis*. The larvae of *Teladorsagia* are therefore likely to be able to
result in an early pasture contamination with infective stage larvae [108]. That may explain why it was the only genus isolated from grass samples in May in the present study. In contrast, the overwintering pasture survival of the more pathogenic *H. contortus* was low, which was absent in the pasture samples until May. This is in agreement with a study on GIN of sheep in Sweden, indicating that only few L₃ of *H. contortus* occur on pasture after 6 weeks of grazing in the spring [147].

Our study showed that pasture contamination in the spring was low but it served as the source of infection both for the young and adult goats. Grass examination revealed two generations of L₃ on pasture related with colder climatic condition during the winter period compared to the study in Spain [148]. The first wave of pasture contamination was observed between July and August, and it was associated with eggs that were shed with the faeces from adult goats between April and June. Most likely these eggs originated from adult worms and that had developed from arrested larvae in the gut mucosa. An increase of infected larvae was observed on pasture. However, it was followed by an increase in FEC, which started in September–October on both farms. This second wave of pasture contamination was the source to arrested stage larvae deposit in GI-tract and origin of the future overwintered population. The ingested L₃ larvae of *H. contortus* in autumn started to develop in early spring and induced clinical signs of II type of hemonchosis with teladorsagiosis in out-wintered yearling goats on Farm A. A sub-acute or chronic form (type II teladorsagiosis) is seen occasionally in housed and out-wintered ruminants in the late winter/early spring, caused by the mass emergence of large numbers of hypobiotic (arrested) larvae, which were acquired in the autumn and overwintered in the gastric glands [42, 71]. The phenomenon of inhibition is multifactorial and promotes nematode survival when the environmental conditions are unfavourable [41] such as darkness and low temperatures [44] but it also occurs in response to the gradual development of required immune responses against nematodes [53]. The tracer test of lambs showed arrested development (100%) of *H. contortus* from July and lower percent of *T. circumcincta* with increasing number of nematodes during the season in Sweden [166]. In our study, a high percentage of arrested *H. contortus* larvae were observed between August and November (53–99%) and also to a lesser extent in early April. Subsequent examination on April 25 showed that ≥95% of total worm burdens were adults and only a low percentage of *H. contortus* and *Teladorsagia* individuals were found in EL₄ stage. The L₃ larvae of *H. contortus* ingested in autumn did not start to develop and induce clinical symptoms until early spring, indicating decreased L₄ larvae development and adult worm reproduction.
Langrova and Jankovska [84] described a high degree of inhibited development in *T. colubriformis* and showed that it was influenced by light and temperature using rabbit model, but in our study *Trichostrongylus* L₄ stage larvae were obtained only in 9%. The L₄ larvae of *Oesophagostomum/Chabertia* in large intestine on November 29 showed low possibility to survive within the host during the winter.

The clinical manifestation of trichostrongylid infection usually may be observed in young goats, as fully expressed immune response against parasites appears at the age of 12 months [63] and they are considered as the most susceptible age group for GIN infection [95]. In the present study, young goats less than 6 months of age were less infected with strongyles, while young goats older than 6 months of age had higher egg excretion when compared to that of adults. However, in some studies no significant impact of age on GIN infection in young and adult goats was recorded [91].

Grazing management had influence on infection level. The feed supplementation or zero-grazing, prevented the animals from the high level of infection. Feed supplementation with red clover and lucerne reduced the intake of infected grass from pasture and enriched the diet with proteins. It has been shown that protein supplementation provides positive effects to improve the host response to nematodes [59]. Additionally, the red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) are characterised as bioactive ingredients, containing some amount of condensed tannins. The legume forages were represented as potential sources for secondary compounds affecting different stages of parasites [55, 59].

The infective larvae of *Ostertagia/Teladorsagia* are able to survive during the winter and persist on pasture until early June [81, 126]. Even in low numbers these larvae may serve as the source of infection in separately grazing young animals. Thus delayed turn-out from middle-June could serve as control measure for prevention of early GIN infections in young stock. The young goats have therefore avoided the most risky period for infection and excreted very low numbers of eggs until the end of the grazing season when compared to the farms where the grazing start in the late April.

*Oesophagostomum, Chabertia* and *H. contortus* infection was lower on farms with zero-grazing and feed supplementation management when compared to those of pasturing regimes. These nematodes are characterized by a low potential to survive unfavourable climatic conditions [108, 166, 126].

The young goats were less infected than adults with *Strongyloides* and *Trichuris* during the summer period presumably due to the short period to higher infection incidence. The goats on open grazing management and tethered goats were less infected with *Strongyloides* than the goats receiving
feed supplement or kept indoors what is in agreement with the findings reported by Manfredi et al. [96]. *Trichuris* eggs were more frequently excreted in spring and autumn in association with indoor period.

Our study showed infection not only with GIN but also with other GI parasites and lung nematodes. *Eimeria* spp. is widespread in goat farms and our study demonstrated 98–100% prevalence of these protozoa in Lithuanian goat farms, which is in agreement with reports from other different countries. In Poland *Eimeria* oocysts were found in 81–100% of investigated goats [7] and in 95% in Estonia [85], 92% in the Czech Republic [82], 100% in Slovakia [160], and 97% in Florida, USA [73]. Goat kids are particularly susceptible to the pathological effects of *Eimeria* infections, especially newly weaned kids kept in large numbers under intensive management conditions [120]. In the present study, the highest oocysts excretion was observed in kids (under 6 months; $P<0.0001$) and young goats (6–12 months; $P<0.001$) when compared to those in adult animals, supporting the findings of several authors [15, 49, 82]. The highest average oocyst count exceeding 20,000 OPG was recorded in kids in the beginning of grazing season. This increase in oocyst production was followed by clinical disease characterized by severe diarrhea and some mortality of kids. This outbreak presumably could be explained by the highest stocking rate combined with poor sanitation on this farm.

In our study, the peak output of oocysts in adult goats was recorded in July-August following the most favourable period with warm and wet environmental conditions for sporulation observed in most of the farms. While oocyst production in adult animals remained comparable throughout the rest of the season, the OPG levels in faeces of young animals was slowly decreasing starting from July–August to the levels comparable to those in adult animals, what could be associated with obtained immunity against coccidiosis.

*Moniezia expansa* infection was detected in 4 of 9 farms (44.4%), but the prevalence was low (4–11.8%). Age and grazing were the risk factors for *M. expansa* what indicates that access to grazing should be considered as a favourable condition for the transmission of this parasite. The incidence of *Fasciola hepatica* is related to environmental conditions, which must be favourable for survival of the intermediate hosts of this liver fluke. Wet pastures on loamy soils without drainage represent a risk factor for liver fluke in ruminants are [18]. In our survey, *F. hepatica* was found on a goat farm where animals grazed near a pond which was a possible source of infection.

The survey on lung worms showed high prevalence (80–85.2%) of *Muellerius capillaris* on goat farms. The similar prevalence (95.5%) was
found in dairy goats in France [24], however lower infection was detected in Norway 31.2% [37] and in Poland 5% [49].

Gastrointestinal nematode infections tend to follow a relatively similar pattern from year to year, therefore we can forecast the infection in the next year and generate the control regimes. If the prevalence of parasites is known and risk factors in a farm are determined it is possible to create effective control programmes with selected drugs. In the absence of appropriate epidemiological knowledge, there are only two bases for anthelmintic administration. The first is to treat suppressively at intervals at or near the length of the pre-patent period of the parasite; the second is to treat therapeutically, whenever clinical approaches, the first is the most effective in the short term in minimising parasite populations and production losses, but has been repeatedly demonstrated both in the field and in computer models to select inexorably for drug resistance in the parasites. The second certainly selects less strongly for resistance, but incurs significant risks of uncontrolled disease or production loss [9]. Alternative anthelmintic administration is before clinical approaches. The determination of this time needs periodical examination of goat faeces. The seasonal pattern on farms can be different depending on heterogeneous treatment schemes on farms, which influence husbandry system and grazing management. The dairy goat farms in our study used preventive treatment during dry period in housing period with therapeutic treatment (if need) for young goats.

The epidemiological survey on two farms showed that deworming of adult goats at the end of November on treated farm significantly reduced the FEC level at the start of grazing compared to goats from untreated farm (P<0.05). Thus, deworming of adults and yearling goats in late autumn reduced the *H. contortus* population in early spring and prevented grass contamination. The high percent of *H. contortus* (31–53%) in faecal cultures of adult and yearling goats showed increased development of this parasite in April–May. If the deworming could not be performed in late autumn it could be implemented in spring before grazing season as prevention against GIN [89].

The study showed high contamination of *L_3* larvae on pasture in July/August and higher FEC in the faeces in August–October. Therefore, the strategic treatment in July was needed to avoid clinical exposure of GI nematode infection during grazing season of young goats. Our investigation on young goats was designed to assess the effect of a treatment administered at 12 weeks after turn-out (on 17 of July) on the permanent pasture in different grazing density farms. The young goats were uninfected with GIN at the start of investigation, while the pasture was contaminated with low
level infective larvae on both investigated farms. The pasture contamination was increasing and the peak was observed in the beginning of August on control group pastures. Also pasture contamination followed an increasing pattern on pastures of treated goats but it was lower compared to controls (P>0.05). The results showed higher pasture contamination on HIG farm and significant difference was between contaminations in control groups with peak in August. As a result, FEC start to increase in August and the peak level in controls was reached in September/October. In the experimental groups of both farms, FEC was similar: 376 EPG and 402 EPG HIG and LIG, respectively. Two weeks after the treatment, young goats did not excrete eggs in faeces; however, after 4 weeks, FEC increased and in HIG farm the increase was faster. The re-infection level after treatment on HIG farm in goats was higher compared to LIG farm in September and October (P<0.05). This difference was related with higher stocking rate on pasture and higher pasture contamination with infective larvae [145]. Comparison of HIG and LIG farms showed that the body weight was lower in both groups of young goats and it was related with higher infection of GIN and with lower amount of grass to each animal. The body condition was poor and farmers had economical losses due to lower production and repeated treatment against GIN. The young goats in HIG farm were treated at the end of investigation because in control group 2 goats were with diarrhoea and 6 with pasty faeces.

### 4.2. Development of anthelmintic resistance in Lithuanian goat farms

Goat industry in Lithuania is predominantly oriented on milk production. This fact was confirmed by questionnaire study where the response was from 100% farmers of dairy goat. Thirty seven farmers keeping from 1 to 4 goats participated at the questionnaire survey. This study exposed risk factors to develop AR in Lithuanian goat farms. Anthelmintics were widely used, 54% of farmers in goat farms used chemotherapy and/or chemoprophylaxis. A control of GIN infections is therefore an important part of livestock management. Unfortunately, a lot of GIN strains have become resistant to anthelmintic drugs and researchers reported all three broad-spectrum anthelmintic groups available for the control of gastrointestinal nematodes of small ruminants (i. benzimidazoles, ii. imidazothiazoles and hydropyrimidines, iii. macrocyclic lactones) worldwide and its impact on sheep and goat farming is dramatically increasing [75, 168].

The questionnaire survey showed that 31.3% of sheep farmers had problems with GI parasites and used anthelmintic treatment. However, only
5.4% of goat farmers reported losses through these parasites. This difference was probably connected with the stocking rate of goats on pasture. The stocking rate on goat farms was 6.3 goats/ha. The average stocking rate on goat farms is predetermined by high stocking rate (13.8/ha) on 5 farms with feed supplementation. By increasing of stocking rate the infection of GI nematodes was observed in greater exposure [145]. Černanska et al. [17] in their study observed the tendencies to treat animals more frequently to avoid parasitic infection. In our study, these tendencies were not observed. The frequency of deworming was 1.2 times/year in goat farms, but not as high as in France – 2.74 times/year [56]. The high treatment frequency is one of the major risk factors for development of anthelmintic resistance [156]. Our survey showed that 11 of 20 goat farms dewormed goats at housing period when the dairy goats were in dry period (November/December). This practice was also determined on goat farms in France [56], Italy [170] and in Norway [36] and may lead to development of anthelmintic resistance.

Under-dosing of anthelmintics leads to development of anthelmintic resistance by surviving with low susceptible nematodes to active substances [72]. The incorrectly estimated animal weight, not corrected drenching gun and insufficient dose reduce the efficiency of anthelmintic. For example, when farmers are asked to estimate the weight of ewes and/or lambs the weights are often under-estimated. It was calculated, that 68% of estimates, for groups of 10–20 sheep, by Australian farmers (n=237) were below the actual weight [13]. Our study showed high danger of under-dosing for goats because 80% of goat farmers in Lithuania estimated live weight by visual appraisal. The dose rate for sheep was used to treat goats in all asked farms and this was comparable to survey performed in Italy [170]. However, Hoste et al. [56] determined that 55% of goat farms in France use double sheep dose for benzimidazoles. Under-dosing is of relevance to the selection of resistance because, when the frequency of anthelmintic resistant alleles is low, under-dosing positively selects for resistance, conversely if resistant allele frequencies are high, selection pressure exerted on heterozygote parasites is less and therefore there is the possibility of the conservation of susceptible alleles [135].

The first survey on AR in Lithuanian goat farms showed the existing resistance of nematodes to anthelmintics. This may be predetermined by faults in treatment management and absence of quarantine for new animals in the farm. In the study of Bartley [11], 12% of the respondents still failed to administer any treatment to new animals, more worryin is the fact that of those who administered quarantine treatment 75% administered only a BZ or an IVM. The importation of resistance alleles with new stock is probably a major contributor to the spread of anthelmintic resistance [28].
In our study, 85% of farmers did not rotate anthelmintic between the years and this also may have contributed to higher AR. Due to this, the exclusive treatment of lambs with an ineffective anthelmintic increased the frequency of BZ resistant worms from 25% to 80% within two years compared to an increase to 50% in lambs alternately treated with BZ and LEV [136].

Macrocyclic lactones (ML) were most frequently used anthelmintic class against GI nematodes on goat farms. This can be related with the broad assortment of products in Lithuania and faster administration, easier dosage and efficiency against ecto-parasites. As it was expected, AR to ivermectin was determined in goat farms in high percentage. In our study, AR to ivermectin was found on all examined farms (N=9). For monitoring ivermectin resistance in field studies it is recommended to use higher discriminating dose (21.6 ng/ml) of ivermectin aglycone to avoid the misdiagnosis of susceptible *Teladorsagia* and *Trichostrongylus* as resistant. Meanwhile, the threshold discriminating dose 10.9 ng/ml should be considered when the L₃ stage of *H. contortus* prevail in the sample [34]. Benzimidazoles were not common anthelmintics for goat farmers in Lithuania. The questionnaire survey showed that 4 farmers from 20 (20%) used benzimidazoles. Resistance by MALDT was detected in two farms where L₃ larvae were detected in the threshold discriminating dose 0.04 µg/ml. The resistance to levamisole was detected only in two farms when the criterion was the use of MIC 2 µg/ml.

In Europe, AR to macrocyclic lactones has been reported from Scotland [71] Germany [132] and Switzerland [4]. Benzimidazoles resistance has been documented from western France [23], northern Italy [170] and Switzerland [134]. The resistance to levamisole was found in France [25], Slovakia [158], and Denmark [104]. The resistance to one or more of the broad-spectrum anthelmintics including macrocyclic lactones has been reported for goat nematodes [52, 134]. Our study showed resistance to IVM and BZ on two farms and IVM with LEV on two farms as well.
CONCLUSIONS

1. The study showed a mixed infection of nematodes in goats in Lithuania. The dominant genus was *Teladorsagia* spp. However also there were determined: *Trichostrongylus* spp., *Oesophagostomum* spp., *Chabertia* spp., *H. contortus*, *Strongyloides* sp. and *Trichuris* spp.

2. The infection of young goats with gastrointestinal nematodes was significantly higher compared to adult goats.

3. Zero-grazing and supplement feeding reduced FEC in the faeces compared to open-grazing and tethering managements (P<0.05).

4. The strategic treatment of young goats on mid-July was effective to control nematodes on high and low grazing intensity farms. It showed lower (P<0.05) faecal egg counts and higher body weight (P<0.05) in treated young goats compared to controls.

5. The biggest farmers’ mistakes of control, which can influence to development of anthelmintic resistance, were under-dosing based on visual appraisal of animal weight and used lower (sheep) dose for goat treatment.

6. The *in vitro* survey of anthelmintic resistance showed resistance to ivermectin in all investigated goat farms (100%), to benzimidazoles in 2 farms of 9 (22%) and to levamisole in 2 farms of 8 (25%).
PRACTICAL RECOMMENDATIONS

1. Due to high prevalence of gastrointestinal nematodes in goat farms, for the determination of infection level it is recommended that farmers collect and send faecal samples to laboratory in late June or beginning of July and repeatedly in October.

2. Special attention should be focused on increasing AR in goat farms. The veterinarians and farmers should dose anthelmintics according to the heaviest animal in group and use double dose of anthelmintics for goats as recommended to sheep.

3. Veterinarians and farmers should be more informed about GI parasites and their control measures. It is particularly important for commercial farms, where goats on pasture are kept in high density.
**SUMMARY IN LITHUANIAN**

**ĮVADAS**

Intensyvėjant žemės ūkiui ir didėjant auginamų gyvulių skaičiui vis dažniau pastebimos parazitų sukeltos sveikatos problemas. Šis ypač pasireiška stambiausiu smulkioju atrajotojų ūkiuose, kur virškinamojo trakto (VT) parazitai padaro didelių nuostolių [30]. Per pastaruosius penkerius metus Lietuvoje pastebima smulkiojų atrajotojų, t. y. avių ir ožkų, skaičiaus augimo tendencija. Per šį laikotarpį ožkų padaugėjo 24 proc. – 2014 metais jų užregistruota 9,3 tūkstančio [1]. Virškinamojo trakto nematodai daro neigiamą įtaką ožkų sveikatingumui, todėl net ir esant subklinikinėms susirgimams gali gali išauginti sveikatos problemas visuomenei [115].

Ypač ženklūs ekonominiai nuostoliai pastebimi dėl atrajotojų šiuo metu net vakarų Europoje, ožkų nelaimėje, o ūkininkai dėl sumažėjusios produktyvumo ir sumažėjusios produkcijos patiria ekonominius nuostolius [115].

Šis parazitas šimtmečiais pastebėtas visame pasaulyje, dėl to jis yra didžiausia parazitų grupė, o ožkų išgaudų terminas gyvūnūnų šaltinio yra H. contortus. Šis parazitas yra plačiai paplitęs šilto klimato regionuose, kur patiriami didelės ekonominės žemesnės ir medžioklės žemesnės, todėl net ir esant subklinikinėms susirgimams gali išauginti sveikatos problemas visuomenei [103, 152].

Pastaruoju metu dėl globalaus klimato šiltėjimo H. contortus pradėjo plisti ir vidutinio klimato zonoje, t. y. Vakarų Europoje, Baltijos bei Skandinavijos šalyse [166, 89, 37].

Nustatyta, kad pastoviai užregistruotas aukštas vidutinio oro temperatūros ir pagausėjusios plūdinės šaltinių kiekio [16].

Ilgą laiką tyrimų rezultatai ir praktiniai nurodymai buvo taikomi ir ožkininkystėje. Tik pastaraisiais metais atsirado pranešimai apie šį parazitą ir paveldėti gali tik avių ir ožkų išgaudų terminas, kur yra H. contortus. Šiame parazite gali būti svarbių evoliucinių skirtumų, ypač tarpavių ir ožkų mitybos ir pasirenkant pašarą. Skiriasi ir šių gyvūnų atsparumas parazitų atsparumas užkariautosioms parazitų ir malą, tačiau šie gryniąti visiškai atsparūs ir mažiausiai žmogaus agentūrai [63]. Ožkos yra mažiausiai atsparūs parazitų atsparumo [68]. Be to, šių gyvūnų pasiūlymo organizme yra skirtos antibiotikinių preparatų farmakokinėtika ir to pasekė su šiais įtakos efektyvumas [20, 39, 122].

Kad būtų pasiekta reikalinga preparato veiksnio koncentracija kraujo plazmoje, dėl greitesnės medžiagų apykaitos bei antibiotikų greitesnio metabolizmo ožkoms reikalinga didesnė šių preparatų dozė. Ši dozė nėra galioti užtikrinti artimiausius laikus ir reikalingas preparatų paveldėti gali tik avių ir ožkų išgaudų terminas, kur yra H. contortus. Šiame parazite gali būti svarbių evoliucinių skirtumų, ypač tarpavių ir ožkų mitybos ir pasirenkant pašarą. Skiriasi ir šių gyvūnų atsparumas parazitų atsparumo užkariautosioms parazitų ir malą, tačiau šie gryniąti visiškai atsparūs ir mažiausiai žmogaus agentūrai [63]. Ožkos yra mažiausiai atsparūs parazitų atsparumo [68]. Be to, šių gyvūnų pasiūlymo organizme yra skirtos antibiotikinių preparatų farmakokinėtika ir to pasekė su šiais įtakos efektyvumas [20, 39, 122].

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informaciniuose lapeliuose tokiu informacija nenurodyta. Taigi atliekant ožkų VT parazitu kontrolę dažnai naudojamas nepakankamos arba klaidingos antihelmintikų dozės.

Apklausos, atliktos įvairiose Europos valstybėse, rodo, kad dėl klaidingai atliekamos ožkų parazitu kontrolės labai dažnai vystosi nematodų atsparumas antihelmintikams (NAA). Ožkų parazitu kontrolės ir NAA tyrimai, atlikti Norvegijoje [36], Danijoje [92, 93], Slovakijos Respublikoje [17], Prancūzijoje [56] ir Ispanijoje [111, 119], pademonstravo, kad gydoma per dažnai, per mažomis preparatų dozėmis, neatliekama šių preparatų rotacija [92, 125, 155]. Tai didelė problema, nes apie NAA smulkuių atragotojų ūkiuose informuoja įvairios Europos šalis − Norvegija [35], Švedija [54], Lenkija [6], Jungtinė Karalystė [10, 99], Vokietija ir Šveicarija [133, 134], Prancūzija [23], Ispanija [97], Graikija ir Italija [48].

Lietuvoje tiriant NAA atlikti pavieniai sporadiniai tyrimai. Nustatyta, kad kiaulių ūkiuose gydant VT nematodus i vermektino efektyvumas (IVM) buvo 90,5–100,0 proc., levamizolio (LEV) − 84,0–100 proc. [141]. Be to, nustatytas 86 proc. veiksmingumas gydant žirgus fenbendazolu (FBZ) nuo strongilų [164]. Tuo tarpu ožkų VT parazitų paplitimo bei NAA tyrimų anksčiau Lietuvoje atlikta nebuvo.

**Darbo tikslas ir uždaviniai**

Disertacinio darbo tikslas – atlikti įvairaus amžiaus ožkų virškinamojo trakto (VT) parazitu epidemiologinį tyrimą Lietuvoje, taip pat ir nematodų antihelmintinio atsparumą (NAA) paplitimą ir nustatytė efektyvias gydymo schemas reguliuojant ožkų VT nematodų populiaciją ganymo sezono metu.

**Darbo uždaviniai:**

1. Ištirti virškinamojo trakto nematodų paplitimą bei sezoninio užsikrėtimo gausumo kūrimą ožkų fermose Lietuvoje;
2. Nustatyti svarbiausius rizikos veiksnius, darančius įtaką ožkų užsikrėtimo virškinamojo trakto nematodais intensyvumui;
3. Ištirti ir palyginti profilaktinio gydymo programos efektyvumą su skirtinio ganymo tanių ožkų fermose;
4. Išanalizuoti ožkų ūkiuose taikomas prevencijos programas prieš nematodus;
5. Taikant in vitro nustatymo metodikas Lietuvos ožkų ūkiuose, nustatyti nematodų atsparumą antihelmintikams.
Darbo mokslinis naujumas ir praktinė reikšmė

Pirmą kartą Lietuvoje buvo ištirtas ožkų VT parazitų paplitimo mastas ir nematodų epidemiologija. Nustatyti ožkų VT didžiausio užsikrėtimo parazitais laikotarpią, pritaikytos ir paruoštos profilaktinės bei gydomosios priemonės. Taip pat pasiūlyta ir praktikoje pritaikyta efektyvi VT parazitų gydymo ir profilaktikos schema įvairaus amžiaus ožkoms. Mūsų šalyje buvo pirmą kartą atlikti nematodų atsparumo antihelminthicams (benzimidazoliams, ivermektiniui ir levamizoliui) in vitro tyrimai, kurie parodyti antihelminthicio rezistenciškumo egzistavimą ir papildė turimas žinias apie šias problemas Europoje.

Remiantis atliktu tyrimų rezultatais, ūkininkams ir veterinarijos gydytojams parengtas lankstinukas apie ožkų VT parazitų pasireiškimą ir jų kontrolės metodus (5 priedas).

TYRIMŲ METODAI IR ATLIKIMAS

Tyrimai atlikti LSMU Veterinarijos akademijos Užkrečiamųjų ligų katedros Parazitologijos laboratorijoje 2010–2014 metais. Tyrimai planuoti ir atlikti trimis etapais (1 pav.).

1 pav. Tyrimų schema
Ūkių pasirinkimas rizikos veiksniams analizuoti

Rizikos veiksniams analizuoti fermos buvo parenkamos pagal dydį (ne mažiau kaip 10 suaugusių ožkų) ir ganymo tipą. Norėdami palyginti skirtingus ganymo tipus, į tyrimą įtraukėme keturias pieninių ožkų fermas: laisvo ganymo (A), pririštų (B), besiganančių, tačiau papildomai šeriamų pjauta žole (C) ir uždario laikomų ožkų (D) (1 lentelė, straipsnis I). Tyrimas atliktas periodiškai imant išmatų mėginius kas mėnesį ganymo laikotarpiu (gegužę–lapkritį) 2011 ir 2012 metais. Ūkiuose A ir B ganymo laikotarpis trunka nuo balandžio mėn. pabaigos iki lapkričio mėn. pradžios. Ūkyje A atjunktyti jaunikliai ganomi atskirame aptvare; ūkyje B jaunikliai ganosi laisvai toje pačioje ganykloje kartu su suaugusiomis ožkomis, kurios rišamos grandine ir perkeliamos vieną kartą per dieną; į tą pačią ganymo vietą grįžta po 6–8 savaitėse. Ūkyje C suaugusios ožkos išginamos gegužės viduryje, o jaunikliai paliekami tvarte nuo birželio viduryje iki rugpjūčio mėn. pabaigos su galimybe pasivaikščioti atskiroje aikštelėje 5 val. per dieną. Šiuo laikotarpiu jaunikliai ir suaugusios ožkos buvo šeriami šienu ir šviežia dobilų arba liucernos žole, koncentratais (grūdų miltais). Ūkyje D visos ožkos nuolat laikomos tvarte, suskirstytame į aptvarus, kuriuose po 10–15 gyvūnų. Jos buvo šeriamos šienu arba šviežia žole, malties ir mėtų grūdų, išspaudų ir daržovių mišiniu (morkų, burokelių, obuolių).

Antihelmintinis gydymas profilaktiškai arba gydymo tikslu buvo atliekamas A ir C ūkiuose. Ūkyje A pasireiškės Eimeria spp. infekcijos klinikiniams požymiams (viduriavimas ir keli gaišimo atvejai), taikytas gydymas toltrazurilu (Baycox®Bovis).

Ūkių atranka virškinamojo trakto nematodų epidemiologiniam tyrimui


Prieš pradedant tyrimą ožkos nebuvos gydytos 4 mėnesius. Bandymo pradžioje atsiktinės atrankos būdu pagal svorį ir lytį (pateles) atrinktos melžiamos ožkos (N=15) ir jaunikliai (N=10). Tyrimo metu išmatų mėginiai buvo imami kas dvi savaites iš tų pačių individų. Dehelmintizacija įvykia A
buvo atliekama ivermektino preparatų Ivomec® 1 proc., po 0,3 mg vienam kilogramui kūno svorio spalio 15 d. jaunikliams ir lapkričio 21 d. suaugusioms melžiamoms ožkoms.

**Ūkių atranka virškinamojo trakto nematodų kontrolės tyrimui**

Norėdami palyginti gydymo efektyvumą ir ekonomiškumą didelio (25 ožiukai/ha) ir mažo (4 ožiukai/ha) ganymo tankio ūkiuose, pasirinkome du Čekų baltosios veislės ūkius, kuriuose ganytoji laikotarpis trunks nuo balandžio pabaigos iki spalio pabaigos. Tyrimas pradėtas 2012 metų balandžio 24-ają – pirmąją ožiukų ganiavos dieną ir truko iki ganiavos pabaigos – spalio 15 d. Išmatų ir žolės mėginiai buvo imami kas dvi savaites, ožiukai buvo sveriami ir vertinama jų išmatų konsistencija. Tyrimas pradėtas su atjunkytais (3 mėn.) panašaus svorio ožiukais, 20 ožiukų kiekvienoje ūkio formoje, iš kurių 10 patelių ir 10 patinėlių. Ūkių įgaivinimui buvo naudojama Panacur® granulės 10 mg/kg kūno svorio. Dėl augalų užsikrėtimo lygio, tyrimo pabaigoje (spalio viduryje), visi ožiukai didelio ganymo intensyvumo ūkyje buvo gydomi ivermektinu (Ivomec® 1 proc.) po 0,3 mg/kg kūno svorio.

**Apklausos organizavimas**


**Ūkių atranka nematodų atsparumo antihelmintikams tirti**

Visi ūkiai išštirti dėl atsparumo benzimidazoliams ir ivermektinui lervų vystymosi agare testu (MALVT), tačiau dėl atsparumo levamizoliui ir benzimidazoliams kiaušinėlių nėrimosi testu (KNT) dėl per mažo kiaušinėlių kiekio ištirti tik penki ūkiai.

**Parazitologinių tyrimų atlikimo technika**

**McMaster metodas**

Modifikuotas McMaster metodas skirtas nustatyti virškinamojo trako parazitų kiaušinėlių skaičių [118]. Išmatų mėginiai iš tiesiosios žamos imami į vienkartinis maišelius ir vežami į laboratoriją. Iki ištirimo laikomi ne ilgiau kaip penkias dienas +4 ºC temperatūroje. 4 g išmatų sumaišoma su 56 ml vandens, išstrinama mentele ir paliekama 30 min. Išmatų suspenzija peršilinojama per marę ir 10 ml pilama į centrifuginį mėgintuvėlį, kuris 7 min. centrifuguoja 1200 apsisukimų per min. greičiu. Nupilamas viršutinis sluoksnis ir ant nuosėdų užpilama cinko chlorido flotacinio tirpalo (tankis 1,4) iki 4 ml žymos. Pastero pipete išmaišoma ir užpildoma McMaster kamera. Kiaušinėliai skaičiuojami šviesiniu mikroskopu naudojant 10x10 padidinimą. Gauti rezultatai dauginami iš 20, ir gautasis skaičius yra kiaušinėlių kiekis 1 g išmatų (KSG), paklaida − 20 kiaušinėlių.

**Lervų kultūrų paruošimas**

Iš kiekvienos tiriamosios grupės ožkų buvo imamas mėginys lervų kultūroms ruošti. Trys vienodi mėginiai ruošti imant po 1g iš kiekvieno individuo. Sumaišyta su nedideliu kiekio vandens ir vermikulitu. Indelis su mišiniu paruoštas pagal Henriksen ir Korsholm metodą [51]. Indelis inkubuoti +24ºC temperatūroje 10–14 dienų. Po inkubacijos indeli su lervomis ruoštas Bermano metodu. Po 24 val. surinktas migravusios L₃ lervos ir 100 invazinių lervų identifikuotos pagal morfologinius skirtumus [90, 154].

**Invazinių lervų rinkimas iš žolės mėginų**

išdžiovinta, o rezultatai skaičiuoti L₃ lervų 1 kg sausos žolės.

**Poskerdiminis tyrimas**


**Kiaušinėlių rinkimas iš išmatų**

Kiaušinėliai NAA tyrimui buvo renkami iš 7–15 ožkų, vyresnių nei 6 mėn. amžius iš šviežių išmatų. Iš kiekvieno mėginio paimta apie 6–10 g į vieną plastikinį maišelį ir sumaišyta (iš viso 100-150 g). Vėliau mėginiai paruošti anaerobinėmis sąlygomis ir laikyti iki tyrimo ne ilgiau kaip penkias dienas. Laboratorijoje nematodų kiaušinėliai surinkti, sijojant per tris vienas ant kito sudėtus sietus su 250 µm, 100 µm ir 20 µm akutėmis. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į stiklinę, iš kurios supiltys į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguoti 1500 aps. per min. Nuo paskutinio sieto nuosėdos su kiaušinėliais perplauto į mėgintuvėlius su dangteliais ir 2 min. centrifuguot
Lervų vystymosi mikro-agare testas
Lervų vystymosi mikro-agare testas (MALVT) taikytas nematodų atsparumą BZ, IVM ir LEV [29]. Tyrimas buvo atliktas 96 duobučių lėkštelese. Pradinis tiabendazo medžiagos tirpalas lėkšteleje serijiniu skiedimu buvo praskiestas 1:2 su dimetilsulfoksidu (DMSO) ir taip gauta 12 galutinių koncentracijų nuo 0,0006 iki 1,28 μg/ml, o ivermektino aglikono (IVMA) (Sigma-Aldrich®, Vokietija) – nuo 0,084 iki 173,6 μg/ml. Pradinis levamizolio hidrochlorido medžiagos tirpalas lėkšteleje serijiniu skiedimu praskiestas 1:2 dejonizuotu vandeniu iki galutinių koncentracijų, nuo 0,0156 iki 32 μg/ml. Lėkštelese pilta 12 μl veiklosios antihelmintiko medžiagos, užpilda 150 μl 2 proc. +45°C temperatūros Bacto agaro. Kai agaras sukietėja įpilda 10 μl mišinio, kuris susideda iš amfotericino B, mielų ekstrakto ir kiaušinėlių suspensijos (galutinis kiaušinėlių kiekis duobėje buvo 30–50). Kontrolinėse lėkštelese buvo naudojamas ne antihelmintikas, o tik DMSO (1,6 proc.) TBZ ir IVMA lėkštelese, o dejonizuotas vanduo LEV lėkštelese. Lėkštelės buvo inkubuotos 7 dienas +25°C temperatūroje. Procesui sustabdyti buvo naudojamas 0,5 Liugolio tirpalas. Visi kiaušinėliai, pirmos, antros ir trečios stadijos lervos suskaičiuojamos, prieš tai perskeliant duobutės turinį ant objektinio stiklolio.

Kiti tyrimai
Oro sąlygų duomenys

Klinikiniai stebėjimai
Tiriant gydymo efektyvumą visi jaunikliai buvo sveriami.
Statistinė analizė


Norint nustatyti LD50 ir LD99, duomenys buvo analizuojami pagal logistinės regresijos modelį, naudojant „Microsoft Excel“ programinę įrangą (versija 2010).

Skirtumas laikytas statistiškai reikšmingu, kai p reikšmė <0,05.

TYRIMŲ REZULTATAI IR JŲ APTARIMAS

Ožkų virškinamojo trakto nematodų epidemiologinė situacija ir gydymo efektyvumas

VT nematodų paplitimo tyrimai atlikti devyniuose ožkų ūkiuose. Nustatyta, kad nematodais buvo užsikrėtę 98,9 proc. suaugusių ožkų ir 93,4 proc. jauniklių. Palyginti su Norvegija, kur nustatyta 61,1 proc. užsikrėtusių ožkų [37], Lietuvoje nematodai paplitimą labiau. Tai galėjo sąlygoti parazitams palankesnės klimato sąlygos. Strongyloides papillosus rastas visuose ūkiuose ir paplitimo mastas svyravo nuo 15,4 iki 70,6 proc. Mažiau aptikta Trichuris sp. ir Capillaria spp. nematodų (1 lentelė).

I lentelė. Kiaušinėlių skaičius suaugusių ožkų ir jauniklių išmatose

<table>
<thead>
<tr>
<th>Nematodai</th>
<th>Paplitimas, proc.</th>
<th>Suaugūsios Ožkos</th>
<th>Jaunikliai Ožkos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vidurkis±SD</td>
<td>Ribos</td>
<td>Vidurkis±SD</td>
</tr>
<tr>
<td>Trichostrongilida</td>
<td>96,8</td>
<td>969,9±1213</td>
<td>9460</td>
</tr>
<tr>
<td>S. papillosus</td>
<td>33,1</td>
<td>52±160</td>
<td>900</td>
</tr>
<tr>
<td>Trichuris</td>
<td>22,7</td>
<td>18±38</td>
<td>200</td>
</tr>
<tr>
<td>Capillaria</td>
<td>2,6</td>
<td>4±5</td>
<td>40</td>
</tr>
</tbody>
</table>

Iabiausiai paplitusios visuose tirtuose ūkiuose, tačiau viename ūkyje vyraujantis nematodas buvo *H. contortus* (2 pav.). Mūsų tyrimų rezultatai atitinka kitų tyrėjų duomenis Norvegijoje [37], Prancūzijoje [24], Graikijoje [110] ir Ispanijoje [150].

![Diagrama](attachment://diagram.png)

**2 pav. Virškinamojo trakto nematodų paplitimas Lietuvos ožkų ūkiuose**

Epidemiologinis tyrimas, atliktas dviejuose ožkų ūkiuose (A ir B) ištisus metus, parodė sezoninę užsikrėtimo virškinamojo trakto nematodais kaitą (Straipsnis II). Tyrinio pradžioje, pavasarį, ganyklų žolė nebuvo gausiai užkrėsta invazinėmis nematodų lervomis. Išėję, kad žiemos metu dažnis lervų žūsta, o išgyvenusios eikvoja savo maisto medžiagas, todėl jų išgyvenamumas pavasarį yra ribotas. Tačiau suaugusieji gyvuliai, kurie iššykią kiaušinėlius į aplinką, papildomas infekcijos šaltinis, ypač jaunikliams, besiganantiams kartu su suaugusiais. Mūsų tyrimo metu išgintos ožkos nebuvo gausiai užsikrėtusios VT nematodais (<500 kiauš./g), tačiau nuo gegužės mėn. užsikrėtės palaipsniui didėjo ir didžiausias išsiskirtų kiaušinėlių kiekis abiejose ūkiuose nustatytas rugsėjo mėn.

Palyginus su negydyto ūkio B ožkų iššykiamu kiaušinėlių kiekiu, ūkyje A atlikta gydyma labai efektyvia mėnesį ženkliai sumažino kiaušinėlių skaičių tvartiniu laikotarpiu. Jauniklių iššykti į ganyklas, nebuvo užsikrėtės VT nematodais, tačiau jų užsikrėtės palaipsniui didėjo, ypač ūkyje A, kuriame ganymo tankis buvo didesnis. Jauniklių išmatose kiaušinėlių pagausėjimas ūkyje A nustatytas rugsėjo–spalio mėnesiais (apie 4000 kiauš./g), o ūkyje B pagausėjusių išmatose kiaušinėlių ženkliai mažiau
(p<0.001) – spalio mėn.


Prevencinis gydymas antihelmintikais, atliktas ūkyje A tvartinio laikotarpiu (lapkričio–lapkričio pavasarį), ženkliai sumažino išskirtų kiaušinėlių kiekį sekančio ganymo pradžioje palengvinti su ūkiu B, kur gydymas atliktas nebuvo. Po gydymo tvartinio laikotarpiu ožkų nematodais buvo užsikrėtę mažai ir H. contortus rūšies nematodų nenustatyta. Ūkyje B tvartinio laikotarpiu taip pat nustatyta žiemos užsikrėtimo lygis, tačiau išmatų kultūrų tyrimas parodė, kad 1–4 proc. sudarė H. contortus. Ištirta, kad balandžio mėn. šių parazitų išskiri amų kiaušinėlių padidėjo iki 38 proc. negydytų ožkų ūkyje, o tai buvo ženkliai daugiau palyginti su gydytų ožkų ūkiu rezultatais (p<0.05).

52–80 proc.), *Chabertia ovina* – 34 proc. (95 proc. PI: 19–49 proc.),
*Trichuris ovis* – 1 proc. (95 proc. PI: 0–3 proc.).

Rizikos veiksnių analizės tyrimas, atliktas keturiuose ožkų ūkiuose
parodė, kad jauni (6–12 mėn.) ožiukai buvo intensyviau užsikrėtę
trichostrongilais palyginti su jaunikliais iki 6 mėn. ir suaugusiomis ožkomis
(p<0,01). Literatūros duomenimis, amžius yra reikšmingas užsikrėtimo
parazitais veiksny, kadangi jauni ožiukai iki 12 mėn. neturi galutinai
susiformavusio imuninio atsako prieš helmintus [63]. Dėl šios priežasties
galimi ūmūs trichostrongiliozės klinikiniai požymiai [95]. Tai kad ožiukai
palyginti su suaugusiomis ožkomis *Strongyloides* ir *Trichuris*
nermatodais užsikrėčia rečiau (p<0,05) galima paaiškinti trupesniu kontakta
laikotarpiu su galimais šių nermatodų platintojais ir aplinka. Mūsų atlikti
tyrimai parodė, kad ganymo būdas daro ženklinių įtaką
invazijos mastui [95]. Ūkio C įtaka įvairias VR nematodų
užsikrėtę trichostrongilais palyginti su besigančiomis laisvai
palyginti besigančiomis ožkomis (ūkis B) (p<0,05). Ūkio C, kur ožkos
buvo šeriamos dobilų ar liucernos žole
mažiau užsikrėtusios trichostrongiloidais
palyginti besigančiomis laisvai (ūkis A) ar
prasistomis ožkomis (ūkis B) (p<0,05). Ūkio C, kur ožkos
buvo šeriamos dobilų ar liucernos žole
mažiau nematodų lervomis užkrėstos žolės pateko į VT, o
baltymų priedas
racione turėjo įtakos didesnių nematodų
imuniniams atsakams prieš nematodas [59].

Sudėtingų tyrinio rezultatų, kai
invazinių lervų skaičius žolėje padidėjo liepos–rugpjūčio mėn.,
ųžsikrėtimo
mastas didėjo ir rugsėjo–spalio mėn. VT
nermatodų kiaušinėlių išskirta
daugiausia. Gydytas buvo suplanuotas antrų
metų liepos viduryje.
Palyginimui pasirinkti du skirtingo ganymo tankio ūkiai, ir gydymas taikytas vienu metu. Tiek didelio ganymo tankumo ūkyje A (25 ožiukai/ha), tiek mažo ganymo tankumo ūkyje B (4 ožiukai/ha) iki liepos mėn. antrosios.
pusės kiaušinėlių su išmatomis išskirta nedaug (<400 kiauš./g) (3 ir 4 pav.). Liepos 17 d. (12-tąją ganymo sav.) atliktas gydomųjų grupių dehelmintizavimas naudojant fenbendazolo preparatą „Panacur“. Gydymas ženkliai sumažino išskiriamų kiaušinėlių kiekį ir padidino priesvorius gydomųjų grupių ožiukų palyginti su kontroliniais. Kontrolinių grupių ožiukų išmatų konsistencija kito, rugpjūčio mėn. išmatos buvo minkštos, o rugsėjo–spalio mėn. dalis gyvulių vidurėjo, dėl to nebuvo priesvorių.

**Nematodų atsparumo antihelmintikams vystymasis Lietuvos ožkų fermose**

Atliktoje apklausoje dalyvavo 37 ėkininkai turintys pieninių ožkų ūkius. Nustatyta, kad viename ūkyje vidutiniškai laikoma 17 ožkų, kurios ganomos 4,4 ha plote. Labiausiai paplitęs ganymas pririšus grandine (62,2 proc. ūkių). Kiti ganymo metodai buvo taikomi rečiau – laisvas ganymas (2,7 proc.), ganymas pastoviose (13,5 proc.) ar rotuojamoje (10,8 proc.) ganyklose, o 10,8 proc. ėkininkų ožkų visiškai neganė. Pagal apklauso nustatyta, kad 54 proc. ėkininkų užsikrėtimo VT nematodais kontrolė naudojo antihelmintikus, o vidutinis gydymo intensyvumas buvo 1,2 karto per metus. Dažniausiai ėkininkai gydymą taikė vieną kartą per metus (46 proc.) tvartiniu laikotarpiu (lapkritis–gruodis) (2 lentelė). Panaši praktika taikoma Norvegijoje [36], Prancūzijoje [56] ir Italijoje [168].

### 2 lentelė. *VT nematodų kontrolė Lietuvos ožkų ūkiuose*

<table>
<thead>
<tr>
<th>VT nematodų kontrolės faktorius</th>
<th>Ožkų ūkių (proc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gydymas</strong></td>
<td></td>
</tr>
<tr>
<td>Negydo</td>
<td>17 (46,0)</td>
</tr>
<tr>
<td>1 kartą per metus</td>
<td>15 (40,5)</td>
</tr>
<tr>
<td>2 kartus per metus</td>
<td>5 (13,5)</td>
</tr>
<tr>
<td><strong>Gydymo periodas</strong></td>
<td></td>
</tr>
<tr>
<td>Tvartinio laikotarpui</td>
<td>12 (60,0)</td>
</tr>
<tr>
<td>Tvartinio laikotarpui ir pavasarį</td>
<td>5 (25,0)</td>
</tr>
<tr>
<td>Esant klinikiniams požymiams</td>
<td>3 (15,0)</td>
</tr>
<tr>
<td><strong>Vaistų dozės apskaičiavimas</strong></td>
<td></td>
</tr>
<tr>
<td>Sveriant (pagal sunkiausią)</td>
<td>2 (10,0)</td>
</tr>
<tr>
<td>Apytikriai</td>
<td>16 (80,0)</td>
</tr>
<tr>
<td>Veterinarjos gydotojas</td>
<td>2 (10,0)</td>
</tr>
</tbody>
</table>

Gydymo metu antihelmintikų dozę ėkininkai dažniausiai (80 proc.) nustatė apytikriai apskaičiuodami gyvulio svorį. Tik 10 proc. ėkininkų
apskaiciuodami doze ožkas sverė, o kitų 10 proc. atsakė, kad ožkas gydo veterinarijos gydytojai (dozės parinkimo būdas nežinomas). Mūsų apklausa parodė, kad ukininkai neturi informacijos apie ožkų organizmo ypatybę geriausiai metabolizuoti antihelmintikų veikliąją medžiagą, todėl reikalingos padidintos dozės skiriama nei tikrųjų. Ištirta, kad pagrindinė parazitų kontrolės klaida, kuri daro didžiausią įtaką NAA vystymuisi, buvo per maža terapiinė dozė, dėl to ženkliai mažėjo parazitų gydymo efektyvumas.


Tyrimais nustatyta, kad Lietuvoje ožkoms gydži dažniausiai naudojami ivermektino preparatai, NAA in vitro tyrimo rezultatai parodė, kad visuose tirtuose ūkiuose (N=9) nematodai ivermektinui yra atsparūs (100 proc.). Pagal MALVΔ naudota 21,6 µg/ml ivermektino aglikono kritinė koncentracija, kur rastos L3 lervos, rodė atsparumą. Vidutinis atsparumo lygis (10–25 proc.) nustatytas trisioje ukiniose, o aukštas (>25 proc.) – šešiose iš 9 ūkių (66,7 proc.). Dviejose ukiniose ivermektinai naudotas nebuvo, tačiau atsparumas šiam antihelmintikui galėjo būti atvežtas su naujais gyvuliais.

### lentelė. Nematojų atsparumas benzimidazoliam, ivermektinui ir levamizolui tiriant MALVΔ

<table>
<thead>
<tr>
<th>Ūkio Nr.</th>
<th>BZ</th>
<th>IVM</th>
<th>LEV</th>
<th>Ūkyje naudoti antihelmintikai</th>
<th>Gydymo kartai/metus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>J (-0.04)</td>
<td>A (+21.6)</td>
<td>J (-2.0)</td>
<td>IVM, LEV</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>DJ (-0.04)</td>
<td>A (+21.6)</td>
<td>J (-2.0)</td>
<td>FBZ</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>J (-0.04)</td>
<td>A (+21.6)</td>
<td>J (-2.0)</td>
<td>IVM</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>J (-0.04)</td>
<td>A (+21.6) A (+20)</td>
<td>LEV, FBZ</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>J (-0.04)</td>
<td>A (+21.6)</td>
<td>J (-2.0)</td>
<td>LEV, IVM</td>
<td>2</td>
</tr>
<tr>
<td>6.</td>
<td>J (-0.04)</td>
<td>A (+21.6)</td>
<td>J (-2.0)</td>
<td>IVM</td>
<td>2</td>
</tr>
<tr>
<td>7.</td>
<td>A (+0.04)</td>
<td>A (+21.6)</td>
<td>J (-2.0)</td>
<td>IVM</td>
<td>1</td>
</tr>
<tr>
<td>8.</td>
<td>J (-0.04)</td>
<td>A (+21.6) A (+20)</td>
<td>IVM, FBZ</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>A (+0.04)</td>
<td>A (+21.6)</td>
<td>IVM, LEV, FBZ</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Pastaba: J –antihelmintikams jautrūs; DJ –antihelmintikams iš dalies jautrūs; A – antihelmintikams atsparūs
Atsparumui benzimidazoliams tirti buvo taikyti du metodai – KNT ir MALVT, kurių rezultatai buvo skirtingi. KNT parodė, kad žemas (<10 proc.) atsparumas nustatytas penkių ūkių septynių (71 proc.). Kadangi šis metodas jautresnis aplinkos veiksnių įtakai, dėl kurių kiaušinėliai gali išsinerti anksčiau, nei paveiks veiklioji medžiaga, todėl galutinėi įsvidai formuoti vadovavomės MALVT rezultatais. Vertinant šio testo rezultatus pasirinkta ribinė koncentracija (0,04 µg/ml), kur dviejų ūkių mėginiuose rasta invażinių lervų (3 lentelė). Viename ūkyje nustatytas nedidelis procentas atsparių nematodų (<10 proc.), tuo tarpu antrame ūkyje – vidutinis (10–25 proc.).

Nustatyta, kad VT nematodai levamizoliui buvo jautrūs šešiuose ūkiuose iš aštuonių (75 proc.), tačiau dviejose nustatytas žemas atsparumo lygis (<10 proc.).

**IŠVADOS**


2. Analizuojant rizikos veiksnius nustatyta, kad ožkų jaunikliai buvo gausiau užsikrėtę VT nematodais palyginti su suaugusiomis ožkomis (p<0,05). Ganomos, bet papildomai šeriamo ir neganomos ožkos VT nematodais buvo užsikrėtusios mažiau lyginti su pririštomis bei laisvai besigandomis ožkomis.


4. Ištirta, kad profilaktinis jauniklių gydymas liepos viduryje buvo efektyvus kontroliuojant intensyvius (25 ožiukai/ha) ir mažo (4 ožiukai/ha) ganymo tankio ūkius, nes gydomosiose grupėse palyginti su kontrolinėmis kiaušinėlių rasta mažiau ir didesnis gyvulio kūno svoris (p<0,05).

5. Apklausos metu nustatyta, kad ūkininkai dažnai neteisingai atlieka ožkų virškinamojo trakto parazitų populiacijos kontrolę, o tai gali turėti įtakos antihelmentiniams nematodų atsparumo didėjimui. Skiriama per maža antihelmentiko veikliosios medžiagos dozė, apskaičiuota apytikliai ir naudojama pagal avims parengtas rekomendacijas.
6. Nematodų atsparumo antihelmintikams in vitro MALVT tyrimais nustatyta, kad visuose tirtuose ožkų ūkiuose (100 proc.) rastas antihelmintinis atsparumas ivermektinui. Benzimidazolams atsparumas rastas dviejuose ūkiuose iš devynių (22 proc.), o levamizoliui dviejuose ūkiuose iš aštuonių (25 proc.).

**PRAKTINĖS REKOMENDACIJOS**


2. Gydymo dozę rekomenduojama apskaičiuoti pasvėrus sunkiausią gyvulij grupėje. Ožkoms gydyti reikalingos dukart didesnės anthelmintikų dozės.

3. Siekiant suteikti daugiau informacijos apie ožkų parazitinių ligų gydymo ir profilaktikos efektyvius kontrolės metodus, reikia ūkininkams ir veterinarijos gydytojams organizuoti daugiau mokymų ir seminarų.
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6. Petkevičius S, Stadalienė I. Control of gastrointestinal nematodes in high and low intensity grazing farms by treatment of young goats. 7th Novel Approaches to Control of Helmints of Livestock with a session of the CAPARA COST ACTION – Goat parasite interaction: from knowledge to control. 25–28 March 2013, Toulouse, France.

85
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The impact of grazing management on seasonal activity of gastrointestinal parasites in goats

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Summary

The aim of this study was to examine the impact of grazing management and other risk factors (age, treatment practices) on seasonal activity of gastrointestinal (GI) parasites in goats. Goat flocks naturally infected with GI parasites reared on four Lithuanian farms representing different management regimes were examined during the grazing season in 2011/2012. On three farms the adult goats were grazed in different ways on open pastures (with or without supplementary feeding) or tethered. On one farm all animals were kept indoor (zero-grazing). On each farm, samples were collected at monthly intervals from 13 – 15 adult and 10 kids. The results showed that grazing of adult goats with/without supplementation or kept indoor, had the lowest number of strongyle eggs when compared to those kept on pasture (P<0.05). Delayed turnout and zero-grazing significantly reduced excretion of strongyle eggs but increased the output of oocysts when compared to those grazed on set-stocked pasture together with adult goats. The most prevalent genus on all farms and in both age groups of goats were Teladorsagia spp. This study demonstrates that goats are infected with mixed species of parasites, but proportions of those parasites differed in different grazing management systems. The grazing management, age and season were all major factors that had an impact on GI parasite infection.

Keywords: goats; grazing management; risk factors; nematodes; Eimeria spp.

Introduction

Gastrointestinal (GI) parasites are important pathogens in ruminants including goats. Infection with GI nematodes is still one of the major constraints in dairy goat production (Rusakid et al., 2007), due to the negative effect of parasites on milk production (Alberti et al., 2012). Production losses in young goats are related to clinical signs such as diarrhea, mortality and subclinical effects, causing long-term weight loss and reduced growth (Charrier & Parard, 2012). The risk of infection with parasites (nematodes and coccidia) in goats depends on several factors. One of the main risk factors, which has an influence on the prevalence of parasites, is the prevailing climatic condition. Lithuania is situated in the northern part of the mid-latitude climatic zone, where seasonal fluctuations in the number of trichostrongyle infective larvae is influenced by variations in temperature and moisture on the soil surface (Morgan & Van Dijk, 2012). Eggs of trichostrongyles develop to the infective third larval stage (L3) above a threshold of around 14°C (O'Connor et al., 2006). The temperature from April to October is therefore favourable for development of the free-living stages on pastures also under Lithuanian climatic conditions. Several other factors may also be associated with GI parasite infection namely grazing management (Mądrański et al., 2010), such as farm size, the stocking rate (Thomsen et al., 1996) and the age of animals (Cabaret & Guaicour, 1994; Guaicour et al., 1997; Sarantas et al., 2011). Moreover, physiological condition and genetic line may have the influence on individual immunity to GI parasite infections (Mądrański et al., 2010). Designing and planning of control strategies requires the knowledge on the epidemiological factors that influence the risk for GI parasite infections. However, published information from Eastern Europe on GI parasites in small ruminants from goats is limited. The aim of this study was to determine the most important epidemiological risk factors that influence the level of GI infections induced by parasite egg or oocyst output in goat flocks in Lithuania.

Materials and methods

Study design

The study was performed in four dairy breed goat farms, located in different regions of Lithuania during the grazing
Table 1. The study design

<table>
<thead>
<tr>
<th>Breed</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flock size</td>
<td>Adult</td>
<td>100</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Kids</td>
<td>170</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>Grazing management</td>
<td>Adult</td>
<td>Open</td>
<td>Tethered</td>
<td>Set-stocked</td>
</tr>
<tr>
<td></td>
<td>Kids</td>
<td>Set-stocked</td>
<td>Open with adults</td>
<td>Delayed grazing</td>
</tr>
<tr>
<td></td>
<td>Kids</td>
<td>20 Aug – 10 Oct</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stocking rate (kg/ha)</td>
<td>Adult</td>
<td>320</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Kids</td>
<td>190 – 235</td>
<td>200 – 230</td>
<td>-</td>
</tr>
<tr>
<td>Separated pastures/pens for weaned kids</td>
<td>Yes</td>
<td>No</td>
<td>Yes/No</td>
<td>Yes/Pens</td>
</tr>
<tr>
<td>Feed supplementation</td>
<td>None</td>
<td>None</td>
<td>Clover, lucerne grass, grain</td>
<td>Fresh grass/hay, grain, vegetables</td>
</tr>
<tr>
<td>Number of treatments against GIN/month</td>
<td>Adult</td>
<td>1/Nov</td>
<td>1/Nov</td>
<td>2/May, Oct</td>
</tr>
<tr>
<td></td>
<td>Kids</td>
<td>1/Oct</td>
<td>-</td>
<td>1/Nov</td>
</tr>
<tr>
<td>Treatment of kids against coccidiosis/month</td>
<td>1/May-Jun</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

of 13 – 15 does and 10 kids. The samples were always collected from the same animals at each sampling occasion. The number of nematode eggs (EPG) and oocysts per gram of faeces (OOG) were determined by a modified McMaster technique (Rosering & Nissen, 1998) using zinc chloride (density 1.4) with a sensitivity of 20 EPG per gram of faeces.

Three replicate herbage samples were prepared for each age group per farm by pooling of 1 g of faeces from the animals within the group and mixed with water and vermiculite (Henriksen & Korsholm, 1983). After 10 – 14 days, L3 were obtained for microscopic identification of nematodes to the genus or species level (Van Wyk et al., 2004). Three replicate herbage samples of approximately 400 g of weight were collected from the pastures grazed by goats for the determination of the numbers of nematode larvae. Each herbage sample was collected while walking across the pasture in a W-shaped pattern and retrieving 5 subsamples at every 20 steps. Grass within 20 cm of faecal pellets was avoided. Larvae were isolated as described by Fernandes et al. (2001), counted and results were expressed as the number of L3 per kg of dried grass. Hay and fresh grass samples were not obtained from Farm D as well as grass from outdoor pens on Farm C.

Other analyses

Data on monthly precipitation and average temperature were obtained from meteorological stations situated 7 – 11 km from the examined farms. For comparison the data during 30-year period (1961 – 1990) were obtained from Lithuanian Hydro Meteorological Service.

Parasitological data on EPG/OOG and prevalence of larvae...
were analysed using BMI SPSS Statistics (Version 21). Statistical comparison of quantitative faecal egg counts between farms or months was performed using Repeated Measures Analysis of Variance (ANOVA). The effect of categorical variables as age (suck — under 6 months, young = 6 — 12 months and adult = over 12 months) and grazing management (open-grazing, tethered, set-stocked of young, young grazing with adults, feed supplementation and zero-grazing) assessed for potential association with strongyle egg and Eimeria oocysts in faeces using Linear Mixed Models procedure. The first-order autoregressive (AR1) correlation structure was used by lower Akaike’s Information Criterion (AIC). The effect of various factors on Strongyloides and Trichu- riasis infections were also assessed. The data contained repeated binary measures of infection in goats with Strongyloides and Trichuriasis as dependent variables along with a fixed recording of age (young or adult), grazing management (open-grazing, tethered, set-stocked of young, young grazing with adults, feed supplementation and zero-grazing), season (spring, summer and autumn) and treated with anthelmintics or not before during the study. The Generalized Linear Mixed Models (GEE) were used to fit a repeated measures logistic regression with this data, using the effect model with the first-order autoregressive (AR1) correlation structure, given the lower Independence Model Criteria (QIC) A value of **P < 0.05** was considered statistically significant.

**Results**

**Nematodes**

In the beginning of study the number of strongyle eggs in adult goat faeces was decreasing in both years (Figure 1a). Furthermore it started to increase reaching the peak values in July/August (Farm B), September (Farm A) and October (Farm C). The highest egg excretion was recorded on Farm B (P < 0.001) during the first year and Farm A (P < 0.001) during the second year of study. The lowest egg excretion was recorded on Farm D (P < 0.05) where 43.8 and 60.0% of goats were not infected with strongyles in 2011 and 2012, respectively. In beginning of study the egg excretion in young goats was low on Farms A and B (Figure 1b). Furthermore it started to increase reaching the peak values in September (Farm B) and September/October (Farm A). The highest excretion of eggs was observed in young goats on Farm B (P < 0.05) in 2011, and Farm A (P < 0.001) in 2012. Egg
In the second year of study contamination of grass with infective L3 stage larvae experienced a different pattern. The numbers of larvae were low throughout the season on Farms B and C while it increased in August (P > 0.05) on the pasture grazed by the young animals on Farm A.


**Oocysts**

In total 812 of dairy goat and 560 of young goat samples were examined. Of these 794 (97.8%) and 550 (98.2%) contained *Eimeria* spp. oocysts respectively. The output of oocysts in adult goats on Farms A and B was higher in October/November in both years (Figure 3a), while on Farm C it peaked in August. The continuously elevated output of oocysts in the feces was recorded on Farm D (P < 0.05) when compared to those on Farms A and B. The oocyst counts in young goat feces were low (P < 0.01) of Farm B when compared to those on Farms A, C and D (Figure 3b). In 2011 the increase in oocyst production was recorded in July on all farms with the highest oocyst excretion on Farm C (above 15,000 OPG). In 2012 the higher oocyst excretion was recorded in beginning of study (May to June on Farms A and D).

**Risk factors**

The results from Linear Mixed Model analysis showed higher (P < 0.001) strongyle egg counts in young (6 – 12 months) goats as well as oocyst counts in kids and young goats when compared to those of adult animals (Table 2). The output of strongyle eggs was significantly reduced with zero-grazing management, when compared to those in other systems. However, zero-grazing was associated with increased oocyst excretion when compared to most of other grazing regimes. Only free grazing of young animals together with adults had significant association with decrease in oocyst excretion. The cestode *Moniezia expansa* infection was recorded only in young goats grazed on pastures (data not shown). The impact of significant risk factors for the presence of *Strongyloides* and *Trichuris* was performed (Table 3).
model suggested that young goats were significantly lower infected with *Strongyloides* and *Trichuris* in summer period. The open-grazing and tethered grazing management has decreased the incidence of *Strongyloides* infection when compared to those of zero-grazing. The presence of regular anthelmintic treatments on farms was a significant regressor with *Strongyloides* infection.

**Discussion**

*Teladorsagia* was the most prevalent genus observed in this study irrespective of goat age and farm management. This genus was similarly predominant on goat farms in Norway (Doukle et al., 2013), France (Charrier & Reche, 1992), Greece (Papadopoulos et al., 2003) and Spain (Valcarcel et al., 1999). Under colder laboratory conditions *Teladorsagia circumcincta* reach the infective stage to a larger extent than those of *Trichostrongylus colubriformis* (O’Connor et al., 2006). The larvae of *Teladorsagia* are therefore likely to be able to result in an early pasture contamination with infective stage larvae. That may explain why it was the only genus isolated from grass samples in May in present study. Nematode egg counts in the faeces from the adult goats were elevated in beginning of study and thus could be regarded as the result of spring/post-partum rise. This was presumably associated with resumed development of inhibited larvae in spring due to depressed immunity.

**Meteorological data**

The monthly temperature was comparable on all farms. The highest average temperature was in July (~18.9 – 19.6 °C). In comparison to multi-annual average values the temperature was higher by 1 – 3 °C during the grazing season particularly in June, 2013. The rainfall was lower in 2011 when compared to those of 2012, but still higher than multi-annual average value. The highest precipitation (58.0 – 170.6 mm) was recorded in July and August 2011 and 2012 on all farms.
Table 2. The impact of age and grazing management on strongyle egg and Eimeria oocyst counts in goat faeces

<table>
<thead>
<tr>
<th>Factor</th>
<th>EPG</th>
<th>Beta Estimates (P-value)</th>
<th>95% CI</th>
<th>OPG</th>
<th>Beta Estimates (P-value)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kids (4-6 months)‡</td>
<td>-0.102 (0.001)</td>
<td>-0.428 - -0.393</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young (6-12 months)‡</td>
<td>0.472 (0.002)</td>
<td>0.254 - 0.690</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grazing management</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open-grazing (adults)‡</td>
<td>0.607 (0.001)</td>
<td>0.364 - 0.850</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tethered (adults)‡</td>
<td>0.644 (0.001)</td>
<td>0.379 - 0.909</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set-to-slab (young)‡</td>
<td>0.435 (0.010)</td>
<td>0.203 - 0.667</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open-grazing (young + adults)‡</td>
<td>0.741 (0.001)</td>
<td>0.554 - 0.927</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed supply (2 treatments)‡</td>
<td>0.131 (0.032)</td>
<td>0.067 - 0.294</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

‡ Compared with adult group
* Compared with zero-grazing management

against strongyle nematodes during lactation period (Chan- khan et al., 2003). It can be assumed that adult animals served as the source for pasture contamination with strongyles eggs in spring and early summer leading to increased numbers of infective 1-stage larvae on pastures in July and August. In adult animals the excretion of strongyle eggs markedly increased in August and September, while in young goats - September or October, as the result of increased contamination of pastures in July and September. Results of our study show that FEC in goats and particularly young goats were significantly higher on Farms A and B when compared to those on Farms C and D, where different management including feed supplementation and double anthelmintic treatment or zero-grazing, prevented the animals from the high level of infection. Feed supplementation with red clover and lucerne on Farm C reduced the intake of infected grass from pasture and enriched the diet with proteins. It has been shown that protein supplementation provides positive effects to improve the host response to nematodes (Hoste et al., 2005). Additionally, the red clover (Trifolium pratense) and lucerne (Medicago sativa) are characterized as bioactive ingredients, containing some amount of condensed tannins. The legume forages were represented as potential sources for secondary compounds affecting different stages of parasites (Hoskin et al., 2003; Hoste et al., 2005). The infective larvae of Ostertagia/Trichostrongylus are able to survive during the winter and persist on pasture until early June (Barklinas et al., 2007; Koepmans et al., 2009). Even in low numbers these larvae may serve as the source of infection in separately grazing young animals. Thus delayed turnout could serve as control measure for prevention of early GIN infections in young stock. On Farm C the young goats were turned out and grazed together with the adults in August when pasture contamination has decreased. The kids have therefore avoided the most risky period for infection and excreted very low numbers of eggs until the end of the grazing season when compared to those on Farms A and B, despite of higher grazing intensity. Anthelmintic treatment during the first weeks on pasture significantly reduced the infection level in the adult goats on Farm B but it did not prevented from the increased pasture contamination observed in July. Due to low exposure of adult animals to infective stages by the end of grazing season in 2011 the egg excretion in 2012 was lower when compared to those on Farm A (P < 0.05) and Farm B (P < 0.05).

The classical manifestation of trichostrongylid infection usually may be observed in kids, as fully expressed immune response against parasites appears in the age of 12 months (Hoste et al., 2010). In the present study kids less than 6 months of age were less infected with strongyles, while young goats older than 6 months of age had higher egg excretion when compared to those of adults. While in some studies no significant impact of age on GIN infection in young and adult goats was recorded (Maione & Masiut, 2002), kids are considered as the most susceptible age group for GIN infection (Mandounet et al., 2003). Kids on Farm B were exposed to highest contamination of pastures in July, which subsequently led to highest strongyle infection in both adult and young animals in 2011. However such situation was markedly improved by anthelmintic treatment administered in November 2011. This resulted in markedly reduced pasture contamination...
Table 3. The incidence of *Strongyloides* and *Trichuris* nematodes eggs in feces and significant risk factors for infection

<table>
<thead>
<tr>
<th>Parasite</th>
<th>No. par/exam. (%)</th>
<th>Factor</th>
<th>Variable</th>
<th>Betas Estimate</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Strongyloides</em></td>
<td>168/1372 (12.3%)</td>
<td>Age</td>
<td>Young*</td>
<td>-1.19</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.19 – 0.61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grazing management</td>
<td>Open-grazing*</td>
<td>-0.64</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.33 – 0.83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tethered</td>
<td></td>
<td>-0.59</td>
<td>0.511</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.33 – 0.91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Season</td>
<td>Summer*</td>
<td>-0.29</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.58 – 0.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treatment</td>
<td></td>
<td>0.82</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.52 – 3.37)</td>
<td></td>
</tr>
<tr>
<td><em>Trichuris</em></td>
<td>148/1372 (12.3%)</td>
<td>Age</td>
<td>Young*</td>
<td>-1.19</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.69 – 0.54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Season</td>
<td>Summer*</td>
<td>-0.69</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.34 – 0.74)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Compared with adult group
*Compared with zero-grazing
*Compared with the reference

(P < 0.05) and a delayed peak contamination observed in September 2012 and lower infection levels in both adult (P < 0.01) and young animals (P < 0.001) in 2012. Pasture contamination and infection of adult and young animals exhibited a different pattern on Farm A. As young animals were set-stocked on the same pasture as both years this resulted in higher accumulation of infective stages on the grass in August 2012 (P = 0.05) when compared to those of previous year on the same pasture. This was followed by proportional increase in egg excretion in young animals manifested in 2012 (P = 0.001).

*Oesophagostomum, Chabertia and H. contortus* infection was lower on farms with zero-grazing and feed supplementation management when compared to those of pasturing regimes. These nematodes are characterized by a low potential to survive unfavourable climatic conditions (Walzer et al., 2004; O’Connor et al., 2006; Sallin et al., 2007).

The young goats were less infected than adults with *Strongyloides* and *Trichuris* during the summer period presumably due to the short period to higher infection incidence. The goats on open grazing management and tethered goats were less infected with *Strongyloides* than did supplemented or kept indoor, which corresponds to the findings reported by Mamedte (2010). *Trichuris* eggs were more frequently excrated in spring and autumn in association with mild weather period. *Moniezia expansa* infection was recorded only in pastured young goats on Farms A and B, which indicate that access to grazing should be considered as a favourable condition for the transmission of this parasite.

In present study the high prevalence (98%) of *Eimeria* infection was recorded in all examined goat farms which is in agreement with reports from other countries. In Poland *Eimeria* oocysts were found in 81 – 100% (Błażecka-Raum, 1999), and in 95% in Estonia (Lassov et al., 2013), 92% in the Czech Republic (Koudela & Boková, 1998), 100% in Slovakia, (Oväk et al., 2004), 97% in Florida, USA (Kahan & Grenier, 2013). Goat kids are particularly susceptible to the pathological effects of *Eimeria* infections, especially newly weaned kids kept in large numbers under intensive management conditions (Ruiz et al., 2006). In the present study the highest oocysts excretion was observed in kids (under 6 months, P < 0.0001) and young goats (6 – 12 months, P < 0.001) when compared to those in adult animals, supporting the findings of several authors (Borgesstre & Decken, 1996; Koudela & Boková, 1998; Goets et al., 2004). The highest average oocyst count exceeding 20,000 OPG was recorded in kids on Farm A in the beginning of grazing season of 2012. This increase in oocyst production was followed by clinical disease characterized by severe diarrhea and some mortality of kids. This outbreak presumably could be explained by highest stocking rate combined with poor sanitation on this farm.

In our study, the peak output of oocysts in adult goats was recorded in July-August following the most favourable period with warm and wet environmental conditions for sporulation observed in most of the farms. While oocyst production in adult animals remained comparable throughout the rest of the season, the OPG levels in faeces of young animals was slowly decreasing starting from August – July to the levels comparable to those in adult animals, which could be associated with obtained immunity against *Eimeria* in goats. The number of *strongyle* eggs and *Eime*
occult oocysts in goat flocks has fluctuated on each examined farm during the grazing season in relation to pasture contamination with infective L1 stage larvae, anthelmintic treatments and immune response in animals.

Acknowledgements

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References


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Seasonal patterns of gastrointestinal nematode infection in goats on two Lithuanian farms

Inga Stadaliene\textsuperscript{1}, Johan Höglund\textsuperscript{2} and Saulius Petkevičius\textsuperscript{1*}

Abstract

Background: This study investigated seasonal changes in naturally acquired gastrointestinal nematode (GIN) infections on two Lithuanian goat farms with different parasite control practices.

Findings: On both farms, nematode faecal egg counts (FEC) and larval cultures were obtained from 1.5 adult and 10 young goats at bi-weekly intervals from April 2012 to April 2013. Goats on farm A were dewormed with ivemectin (0.3 mg/kg body weight) in October/November 2012, whereas the animals on farm B were left untreated. Thirteen young goats were slaughtered in August/November 2012 and April 2013 and worm burdens in the gastrointestinal tract were enumerated. In goats from both farms, Teladorsagia, Trichostrongylus, Cooperia, Oesophagostomum, Chabertia and Haemonchus were the dominant GIN genera. Herbicide contamination with infective third-stage larvae (L3) peaked in July/August and resulted in high FEC in September/October. Parasitological examination at slaughter showed that Teladorsagia spp. and Haemonchus contortus survived the winter, both in the abomasal mucosa as adults and as early fourth-stage larvae (L4). Deworming on farm A significantly reduced FEC, especially of H. contortus, at the start of the grazing period compared with the untreated farm B (P < 0.05).

Conclusions: Goats were heavily infected with several GIN throughout the year. Strategic anthelmintic treatment during housing significantly reduced nematode egg output, in particular by H. contortus, at the start of the grazing season.

Keywords: Goats, Epidemiology, Arrested larvae, Teladorsagia, Haemonchus contortus

Findings

The risk of gastrointestinal nematode (GIN) infections in small ruminants is determined by factors such as climate, level of nutrition, stocking density and management. During the past two years, the goat population in Lithuania has increased by 28%, to 9,300 animals in 2014 [1]. This has led to increased stocking rates on pasture, with associated productivity losses from GIN infections [2].

The effects of GIN are predicted to become more severe due to global warming [3,4]. One such example of the possible influence of ongoing climate change is the increased infection levels of Haemonchus contortus observed in Scandinavia [5,6]. However, as long as anthelmintics are effective, strategic treatments reduce parasite contamination and lower the exposure to GIN associated with increased milk yields in goats [7]. Deworming during housing is the most commonly used control practice against GIN in Norway [8] and it has been shown that it significantly reduces faecal egg counts (FEC) in the next grazing season [9]. Reduced FEC at the start of the grazing period prevents pasture contamination, especially by H. contortus since its free-living stages are sensitive to sub-zero temperatures [3,10]. H. contortus mainly survives the winter inside the host, as arrested forms in the abomasal mucosa [11]. Previous studies on GIN in Lithuanian sheep and cattle have shown high levels of larval inhibition [12,13]. In contrast, information from Lithuanian goats has hitherto been lacking.

This study investigated seasonal fluctuations in GIN on two goat farms in central Lithuania, of which farm A was treated with anthelmintics and farm B was left untreated. Both farms had White Shorthaired Goats. The study took place between April 2012 and April 2013 and the grazing period was from late April until late...
October. During housing, the animals were fed hay/haye
lage, vegetables (sugar-beet, carrots and grain. The kid-
ding period on both farms started in late January and
lasted until late February. Kids were weaned after ap-
proximately 3 months. On farm A, the kids grazed sepa-
rate from the adult goats until October in a paddock
with 500 kg/ha (adults 320 kg/ha), whereas on farm B
they grazed in the same paddock (235 kg/ha).

Mean monthly temperature was similar and peaked
(19.6°C) in July. Compared with the long-term average
(1961–1990), the temperature on both farms was on an
average 1–3°C higher during grazing. Winter tempera-
ture varied on average between −1.0°C and −6.7°C. The highest
level of rainfall was observed in June/July (897.7-
1386 mm).

Anthelmintics had not been used on either farm for
4 months prior to the start of the trial. On farm A, the
young goats received injectable ivermectin (Ivomec® 1%,
0.3 mg per kg body weight) in early October, while the adults
were dewormed in late November. On farm B, all
goats were left untreated throughout the study.

The work was performed in compliance with Lithu-
anian animal welfare regulations (No. B1-866, 2012; No.
XI-2271, 2012) and was approved by the Lithuanian
Committee of Veterinary Medicine and Zootechnics Sci-
ences (Protocol No.07/2010).

Animals in each flock were selected by stratified ran-
dom sampling by sex (female) and body weight, and
categorized as young goats (≤1 year; n = 10) or adults
(>1 year; n = 15). Faeces samples were collected directly
from the rectum at bi-weekly intervals. FEC were per-
formed using a modified McMaster technique with
minimum detection level of 20 nematode eggs per gram
(EPG) faeces [14]. Furthermore, 1 g samples of faeces
from each animal in the same grazing group were pooled
and faecal cultures were prepared to obtain infective
third-stage larva (L3) [15]. Identification to genus or
class level was based on morphological keys [16]. In
addition, triplicate (±400 g) samples of herbage were
collected between May and November 2013 from each of
the paddocks used by the goats on both farms, for deter-
mination of number of L3 [17]. Furthermore, six young
females from farm A and seven from farm B were sent
to the local slaughter-house for slaughtering between 25
August and 25 April 2013 and the viscera of the goats
were collected for parasitological investigation. The ab-
omasum and small- and large intestines were opened for
enumeration and identification of GIN, while the ab-
omasal mucosa was digested and examined for inhibited
stages according to Grønvold [18]. Nematodes were col-
lected and identified to species or genus [19]. Statistical
comparisons of FEC between farms were performed
using Repeated Measures Analysis of Variance (ANOVA)
analysis in IBM SPSS Statistics 21 version. Prevalence of
nematode infections in the gastrointestinal (GI) tract
and standard 95% confidence intervals (CI) were also
calculated. Worm burdens on the two farms were com-
pared using one-way ANCOVA, with P < 0.05 as statisti-
cally significant.

Pasture contamination was low (88–499 L3/kg) at
the start of the study and remained low until late June (Figure 1).
In contrast, from late June, L3 numbers in herbage began to increase, with a peak in late July, in
the paddock used by adult goats on farm A and in the
common paddock on farm B. Two weeks later, in Au-
 gust, there was a peak in L3 pasture contamination in
the paddock grazed by the young goats on farm A. These
peaks in L3 between July and August represented the
first wave of pasture contamination, and must have
originated from nematode eggs shed with the faeces of
adult goats between April and June (Figure 2A). These
parasite eggs most likely originated from adult worms that
had resumed their development from arrested larvae in
the mucosa of adult goats. This infection wave resulted in
subsequent peaks of parasite eggs shed, which were ob-
served in both adult flocks in September. However, the
FEC was much higher on farm A, probably as a result of the
high stocking density. The FEC in young goats on
both farms peaked and reached its highest levels in Sep-
tember/ October 2012 (Figure 2B). However, again FEC

Figure 1 Mean number of L3 stage larvae per kg dry matter of grass on farms A and B.
was significantly higher (P < 0.001) on farm A, probably as a result of the higher grazing intensity compared with farm B. All young goats on farm A were dewormed in October 2012. Although this decreased FEC, it did not prevent the spring rise in FEC, which was observed towards the end of April in the next year. The second wave of pasture contamination in October resulted in re-infection and a source of arrested larvae, creating an overwintering nematode population in the GI tract. During the housing period (November – April), FEC in the young goats gradually increased, followed by a marked rise by the end of April 2013 on both farms.

*Teladorsagia* dominated both in the samples from pasture (42 - 100%) and in faecal cultures (42%) on both farms, confirming results from other European countries [6,20-22]. In addition, *L3* of *Trichostrongylus* (26%),

Table 1 Mean number of worms and proportion (% of total) of *Haemonchus contortus* and *Teladorsagia* sp. development stages in abomasum of young goats at slaughter

<table>
<thead>
<tr>
<th>Date</th>
<th><em>H. contortus</em></th>
<th></th>
<th>Mean total</th>
<th></th>
<th><em>Teladorsagia</em></th>
<th></th>
<th>Mean total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>DL&lt;sub&gt;50&lt;/sub&gt;</td>
<td>EL&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Mean total</td>
<td>Adult</td>
<td>DL&lt;sub&gt;50&lt;/sub&gt;</td>
<td>EL&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>25 Aug</td>
<td>134 (20%)</td>
<td>54 (9%)</td>
<td>515 (74%)</td>
<td>741</td>
<td>4049 (89%)</td>
<td>154 (34%)</td>
<td>908 (12%)</td>
</tr>
<tr>
<td>14 Sep</td>
<td>71 (11%)</td>
<td>0</td>
<td>8325 (99%)</td>
<td>8306</td>
<td>10346 (86%)</td>
<td>451 (35%)</td>
<td>4500 (31%)</td>
</tr>
<tr>
<td>01 Oct</td>
<td>300 (22%)</td>
<td>225 (25%)</td>
<td>251 (53%)</td>
<td>776</td>
<td>4522 (89%)</td>
<td>111 (29%)</td>
<td>183 (40%)</td>
</tr>
<tr>
<td>29 Oct</td>
<td>0</td>
<td>24 (1%)</td>
<td>3656 (99%)</td>
<td>3680</td>
<td>5633 (89%)</td>
<td>74 (1%)</td>
<td>826 (13%)</td>
</tr>
<tr>
<td>20 Nov</td>
<td>239 (26%)</td>
<td>0</td>
<td>2342 (89%)</td>
<td>2581</td>
<td>7166 (88%)</td>
<td>12 (1%)</td>
<td>1026 (11%)</td>
</tr>
<tr>
<td>25 Apr</td>
<td>495 (57%)</td>
<td>0</td>
<td>14 (9%)</td>
<td>495</td>
<td>1083 (96%)</td>
<td>0</td>
<td>46 (9%)</td>
</tr>
</tbody>
</table>

*Note*: circumsinuta and *Te* cibiculata.
Table 2 Mean number of worms and proportion (% of total) of Trichostrongylus and Oesophagostomum/Chabertia development stages in the small and large intestines of young goats at slaughter

<table>
<thead>
<tr>
<th>Date</th>
<th>Trichostrongylus*</th>
<th></th>
<th>Oesophagostomum/Chabertia*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult (n)</td>
<td>Developing (n)</td>
<td>Adult (n)</td>
<td>Developing (n)</td>
</tr>
<tr>
<td>25 Aug</td>
<td>2851 (79%)</td>
<td>1065 (25%)</td>
<td>3919 (16%)</td>
<td>667 (20%)</td>
</tr>
<tr>
<td>14 Sep</td>
<td>13005 (91%)</td>
<td>1000 (9%)</td>
<td>16350 (51%)</td>
<td>130 (17%)</td>
</tr>
<tr>
<td>06 Oct</td>
<td>30174 (37%)</td>
<td>4073 (15%)</td>
<td>39263 (38%)</td>
<td>68 (62%)</td>
</tr>
<tr>
<td>29 Oct</td>
<td>4697 (99%)</td>
<td>87 (9%)</td>
<td>4784 (75%)</td>
<td>20 (8%)</td>
</tr>
<tr>
<td>29 Nov</td>
<td>4962 (99%)</td>
<td>419 (7%)</td>
<td>5381 (79%)</td>
<td>30 (21%)</td>
</tr>
<tr>
<td>35 Apr</td>
<td>8775 (100%)</td>
<td>0</td>
<td>8775 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* T. colubriformis and T. vitr. (13%), Chabertia (11%) and H. contortus (8%) were identified in the faecal cultures. Interestingly, H. contortus was only observed during the grazing period (May-October) on farm A (3 – 35% of the total nematode population) and farm B (8 – 31%). In addition, H. contortus was detected in faecal cultures of young goats from late June and increased between August and October. This was particularly the case on farm A, where H. contortus comprised 16 – 21% of the total nematode population. Similarly, pasture contamination with H. contortus larvae was first detected in mid-May, but was highest between August and October (9 – 50%). This is in agreement with Swedish findings on GIN in sheep [10].

In the GI tract, the following species were identified in the abomasum: Teladorsagia circumcincta 52% (95% CI: 42 – 62%), T. trifurcata 34% (95% CI: 23 – 47%), Trichostrongylus axei 7% (95% CI: 3 – 12%) and H. contortus 7% (95% CI: 1 – 12%). In the small intestine, the species identified were: T. capricola 45% (95% CI: 34 – 56%), T. columbaformis 43% (95% CI: 32 – 52%), T. vitr. 11% (95% CI: 7 – 16%) and Strongyloides papillosus 1% (95% CI: 0 – 2%). The species found in the large intestine were: Oesophagostomum venulosum 65% (95% CI: 52 – 80%), Chabertia ovina 34% (95% CI: 19 – 49%) and Trichuris ovis 1% (95% CI: 0 – 3%). For Teladorsagia spp., adult worms dominated in the abomasum, while for H. contortus early fourth-stage larvae (EL4) dominated (Table 1). Arrested H. contortus were mainly observed between August and November (93 – 99%) but to a lesser extent also in early April (Table 1). Developing larvae (EL4) of H. contortus were found until late October and of Teladorsagia spp. until late November. Subsequent examinations in April showed that ≥95% of the total worm burdens were adults, with only a low percentage of H. contortus (3%) and Teladorsagia (4%) in the EL4 stage. The EL4 of H. contortus ingested in autumn obviously did not start to develop until early spring, resulting in decreased EL4 development and adult worm reproduction. Tracer tests on lambs in Sweden have shown that arrest of H. contortus takes place mainly from July and that T. circumcincta comprises a lower percentage of the increasing numbers of nematodes during the grazing season [11]. The significantly higher numbers of adults than of developing stages (P < 0.05) of Trichostrongylus spp., O. venulosum and C. ovina observed in the tracers indicate that these nematodes survived the winter within the host as adults (Table 2). Deworming of adult goats at the end of November on farm A significantly reduced FEC at the start of grazing compared with the untreated farm B (P < 0.05). After deworming on farm A, H. contortus L3 were not observed in faecal cultures until March 2013 and in April comprised only 2 – 3%. The corresponding proportion of this parasite in faeces of goats on farm B was 1 – 4% during housing, but in April of the next year 38% of the cultured L3 were H. contortus. Thus, deworming of adults and young goats in late autumn reduced the H. contortus population considerably in early spring and thereby prevented pasture contamination. The high percentage of H. contortus (31 – 53%) in faecal cultures of adult and young goats shows that development of this parasite resumed from April–May. If deworming is not performed in late autumn, it could be implemented in spring before turn-out [5].

In conclusion, the Lithuanian goats studied here were infected with a mixture of GIN, in particular Teladorsagia spp. but also several other genera, including the more pathogenic H. contortus. FEC fluctuated in relation to the level of herbage contamination, which varied according to season on both farms. Strategic anthelmintic treatment of adult goats in November significantly reduced FEC, especially of H. contortus.

Competing interests: The authors declare that they have no competing interests.

Authors’ contributions: IS collected and analysed the data, performed the literature review and drafted the manuscript; DP generated the study design; coordinated the experiment and took part in the writing. IS participated in planning and preparing the study design and finalised the manuscript. All authors read and approved the final draft of the manuscript.
Acknowledgements
The authors thank the owners of farms A and B for their participation in the study, veterinary students of the Veterinary Academy, Lithuanian University of Health Sciences, for their help in collection of samples and Dr. Mary McKee for language editing.

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References

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100
ACCESSORIES

Accessory No.1

Questionnaire

ALL DATA WILL BE TREATED AS CONFIDENTIAL
Please mark the answer or write

1. Farm address _________________________________________________________
2. Total area of pasture? ___________ Acres or ___________ Hectares
3. Goat breed(s)? ________________________________________________
4. Annual numbers of goats? Adult: ___________ Young (<1 year): ___
5. Grazing management? 1) Open grazing 2) Tethered 3) Pasture rotation
4) Permanent pasture 5) Zero-grazing
6. Do your young goats grazing with adults? 1) Yes 2) No
7. Do you have other ruminants? 1) Yes 2) No
   i) Cattle  ii) Sheep
8. How often do you treat per year? _______________________________
9. Do you have problem with parasites? 1) Yes 2) No
   i) Nematode  ii) Liver fluke  iii) Tapeworm
10. When do you treat animals?

<table>
<thead>
<tr>
<th>Adult</th>
<th>Young</th>
</tr>
</thead>
<tbody>
<tr>
<td>At housing</td>
<td></td>
</tr>
<tr>
<td>At housing and in spring</td>
<td></td>
</tr>
<tr>
<td>Combination</td>
<td></td>
</tr>
<tr>
<td>Clinical signs/scouring</td>
<td></td>
</tr>
</tbody>
</table>
11. How do you calculate anthelmintic dose?

   1) Weighing (average weight) 2) Visual appraisal 3) By veterinarian
12. Do you move your animals to clean pasture after treatment? 1) Yes 2) No
13. What anthelmintics did you use this year?

   1) Benzimidazoles (Albendazole, fenbendazole) 2) Macro cyclic lactones (Ivermectin)
   3) Imidazothiazole (Levamisole)
14. How often do you change anthelmintics?

   1) Each time you use treatment 2) Two times per year
   3) Annually 4) Longer 5) Never
### Accessory No. 2

**A. Count of Trichostrongylids in young goat faeces (eggs/g)**

<table>
<thead>
<tr>
<th>Farm Nr.</th>
<th>Mean</th>
<th>SD</th>
<th>MIN</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1080.0</td>
<td>237.9</td>
<td>780</td>
<td>1360</td>
</tr>
<tr>
<td>2.</td>
<td>650.0</td>
<td>357.2</td>
<td>260</td>
<td>1200</td>
</tr>
<tr>
<td>3.</td>
<td>296.0</td>
<td>130.7</td>
<td>120</td>
<td>420</td>
</tr>
<tr>
<td>4.</td>
<td>288.6</td>
<td>204.9</td>
<td>60</td>
<td>560</td>
</tr>
<tr>
<td>5.</td>
<td>1912.0</td>
<td>2481.3</td>
<td>440</td>
<td>6320</td>
</tr>
<tr>
<td>6.</td>
<td>433.3</td>
<td>812.6</td>
<td>0</td>
<td>2080</td>
</tr>
<tr>
<td>7.</td>
<td>2260.0</td>
<td>880.0</td>
<td>820</td>
<td>4200</td>
</tr>
<tr>
<td>8.</td>
<td>936.0</td>
<td>235.1</td>
<td>620</td>
<td>1240</td>
</tr>
<tr>
<td>9.</td>
<td>1148.0</td>
<td>488.4</td>
<td>560</td>
<td>1760</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>1000.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B Count of Trichostrongylids in adult goat faeces (eggs/g)**

<table>
<thead>
<tr>
<th>Farm Nr.</th>
<th>Mean</th>
<th>SD</th>
<th>MIN</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>750.0</td>
<td>1011.8</td>
<td>60</td>
<td>3180</td>
</tr>
<tr>
<td>2.</td>
<td>565.3</td>
<td>354.8</td>
<td>80</td>
<td>1200</td>
</tr>
<tr>
<td>3.</td>
<td>740.0</td>
<td>616.1</td>
<td>120</td>
<td>1860</td>
</tr>
<tr>
<td>4.</td>
<td>340.0</td>
<td>261.1</td>
<td>140</td>
<td>920</td>
</tr>
<tr>
<td>5.</td>
<td>3012.5</td>
<td>2794.1</td>
<td>520</td>
<td>9460</td>
</tr>
<tr>
<td>6.</td>
<td>221.3</td>
<td>202.2</td>
<td>0</td>
<td>640</td>
</tr>
<tr>
<td>7.</td>
<td>754.7</td>
<td>432.6</td>
<td>220</td>
<td>1740</td>
</tr>
<tr>
<td>8.</td>
<td>1425.0</td>
<td>1292.5</td>
<td>440</td>
<td>4500</td>
</tr>
<tr>
<td>9.</td>
<td>920.0</td>
<td>800.7</td>
<td>380</td>
<td>2660</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>969.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Accessory No. 3

**Prevalence of GI parasites in goat farms**

<table>
<thead>
<tr>
<th>Farm Nr.</th>
<th><strong>Strongyloides papillosus</strong> positive/tested</th>
<th><strong>Trichuris</strong> positive/tested</th>
<th><strong>Capillaria</strong> positive/tested</th>
<th><strong>Moniezia</strong> positive/tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2/13 (15.4%)</td>
<td>0</td>
<td>0</td>
<td>1/25 (4%)</td>
</tr>
<tr>
<td>2.</td>
<td>11/25 (44%)</td>
<td>8/25 (32%)</td>
<td>0</td>
<td>1/25 (4%)</td>
</tr>
<tr>
<td>3.</td>
<td>3/12 (25%)</td>
<td>3/12 (25%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>12/17 (70.6%)</td>
<td>6/17 (35.3%)</td>
<td>1/17 (5.9%)</td>
<td>2/17 (11.8%)</td>
</tr>
<tr>
<td>5.</td>
<td>2/13 (15.4%)</td>
<td>2/13 (15.4%)</td>
<td>0</td>
<td>1/13 (7.7%)</td>
</tr>
<tr>
<td>6.</td>
<td>5/24 (20.8%)</td>
<td>3/24 (12.5%)</td>
<td>1/24 (4.5%)</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>5/25 (20.0%)</td>
<td>7/25 (28.0%)</td>
<td>1/25 (4.0%)</td>
<td>1/25 (4.0%)</td>
</tr>
<tr>
<td>8.</td>
<td>5/13 (38.5%)</td>
<td>2/13 (15.4%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9.</td>
<td>3/12 (25.0%)</td>
<td>3/12 (25.0%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

---

102
**Accessory No. 4**

**A. Count of Eimeria spp. in young goat faeces (oocysts/g)**

<table>
<thead>
<tr>
<th>Farm Nr.</th>
<th>Mean</th>
<th>SD</th>
<th>MIN</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1556.0</td>
<td>1179.6</td>
<td>480</td>
<td>3420</td>
</tr>
<tr>
<td>2.</td>
<td>4626.0</td>
<td>6865.9</td>
<td>60</td>
<td>21560</td>
</tr>
<tr>
<td>3.</td>
<td>2256.0</td>
<td>1855.9</td>
<td>560</td>
<td>4820</td>
</tr>
<tr>
<td>4.</td>
<td>2260.0</td>
<td>3917.4</td>
<td>120</td>
<td>11020</td>
</tr>
<tr>
<td>5.</td>
<td>3080.0</td>
<td>2384.2</td>
<td>700</td>
<td>6120</td>
</tr>
<tr>
<td>6.</td>
<td>1662.2</td>
<td>3011.5</td>
<td>20</td>
<td>7760</td>
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<tr>
<td>7.</td>
<td>958.0</td>
<td>679.8</td>
<td>240</td>
<td>2580</td>
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<tr>
<td>8.</td>
<td>3824.0</td>
<td>1934.7</td>
<td>1680</td>
<td>6740</td>
</tr>
<tr>
<td>9.</td>
<td>29724.0</td>
<td>62012.8</td>
<td>1020</td>
<td>140640</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>5547.4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B. Count of Eimeria spp. in adult goat faeces (oocysts/g)**

<table>
<thead>
<tr>
<th>Farm Nr.</th>
<th>Mean</th>
<th>SD</th>
<th>MIN</th>
<th>MAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>230.0</td>
<td>95.0</td>
<td>60</td>
<td>300</td>
</tr>
<tr>
<td>2.</td>
<td>1965.3</td>
<td>5436.3</td>
<td>60</td>
<td>21560</td>
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<tr>
<td>3.</td>
<td>1345.7</td>
<td>1429.5</td>
<td>180</td>
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<tr>
<td>4.</td>
<td>286.0</td>
<td>140.6</td>
<td>140</td>
<td>460</td>
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<tr>
<td>5.</td>
<td>2295.0</td>
<td>3190.6</td>
<td>140</td>
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<tr>
<td>6.</td>
<td>241.3</td>
<td>107.3</td>
<td>60</td>
<td>400</td>
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<tr>
<td>7.</td>
<td>441.3</td>
<td>343.6</td>
<td>80</td>
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<tr>
<td>8.</td>
<td>2645.0</td>
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<td>9.</td>
<td>780.0</td>
<td>573.4</td>
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<tr>
<td><strong>Mean</strong></td>
<td><strong>1136.6</strong></td>
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</tbody>
</table>
Svarbu tai, kad ožkos skiriami antihelmintikai yra greičiau biet transformuojami ožkų kaušyje nei avių. Ožkos naudojamos antihelmintikų dozės turi būti 1,5-2 kartus didesnės nei rekomenduojamos avių bei galvijoms.

<table>
<thead>
<tr>
<th>Aktyvumo prieš avylų/ožkų parazitus spektras</th>
<th>BZ</th>
<th>I/T</th>
<th>ML</th>
<th>OP</th>
<th>CLOS</th>
<th>TRBC</th>
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<td>Habronema papillosus</td>
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<td>Storaxis žarnynas</td>
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<td>Oesophagostomum spp.</td>
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<td>Oesophagostomum spp.</td>
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<td>Oesophagostomum spp.</td>
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<tr>
<td>Oesophagostomum spp.</td>
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Kodel ir kuo kokiais paražitois kovoti?

Svarbūs punktai, kuriuos reikia įvertinti prieš pradėdant kovoti su paražitois

- Tiksliausias ir efektyvusios kovos būdas prieš paražitus yra žiant aukštesnį – BŪTINŲ LABORATORINIAI TYRIMAI!
- Atskirinate į mūsų gyvulio grupes ir kategorijas:
  - Išskirtinių statų (įvairių gyvulio, pažeista paviršiu, gorepaviršiu);  
  - Išskirtinių periodų;
  - Produkuojančio įtaką (išdėstymo, aukščio, įvairių paražito);  
  - Įvairių gyvulio ir gyvulio būdų;
  - Visuose ir paauksliųse produkcijose;
- Įspūdžio veikalienį įvertinkite:
  - Įvairiu kortuose;
  - Įvairiu gyvulio pranešimų;
  - Įvairiu gyvulio šaltiniu;
  - Įvairiu gyvulio dienų;
  - Įvairiu gyvulio skaičiu;
- Gyvų kovos paaiškimam:
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ATSKIRŲ/ PARAŽITOŲ LIDINĮ PASIREIKŠKIMO LIKAS

- If you need more information, please provide additional context or specific questions regarding the content of the page. This will help in accurately translating the content. 

Kontrolės priėmų virškinimo trakto nematodijų būdai

- Gyvūnų reguliavimas:
  - Periodinių gyvų ir gyvulio paviršių metų per metus;
  - Gyvulio ir gyvulio šaltinių metų per metus;
  - Gyvulio ir gyvulio pažeidžiantys metų per metus;
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Genetinė selekcija

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ACKNOWLEDGEMENTS

First, I would like to thank my supervisor Prof. Habil. Dr. Saulius Petkevičius for his useful advices, brilliant patience and wonderful personality which made a great contribution to the success of this study. Special thanks go to Dr. Mindaugas Šarkūnas for help to build the experimental plans and to senior technician Vilija Laurinavičiūtė for helping with laboratory work.

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Finally, I thank my husband Osvaldas, sons and all my family for their continuous support and understanding.

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CURRICULUM VITAE

Inga Stadalienė was born in Marijampolė, Lithuania on the 19th of October, 1983. She completed Rygiškių Jonas, Marijampolė gymnasium in 2002. From 2002 to 2008 she studied at the Faculty of Veterinary Medicine, Lithuanian Veterinary Academy where she obtained the Veterinary Medicine Master's degree. From 2008 to 2011 I. Stadalienė worked as a private veterinarian in small animal clinic „Fitoveta ir KO“. In 2010, Inga started the PhD studies at the Department of Infectious Diseases, Veterinary Academy, Lithuanian University of Health Sciences.