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ANTHELMINTIC RESISTANCE IN SHEEP GASTROINTESTINAL NEMATODES IN LITHUANIA: PREVALENCE AND RISK FACTORS ANALYSIS

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CONTENTS

ABBREVIATIONS

AR	–	anthelmintic resistance
BZ	–	benzimidazole
CI	–	confidence interval
DMSO	–	dimethyl sulfoxide
DNA	–	deoxyribonucleic acid
EHT	–	egg hatch test
EPG	–	eggs per gram
FEC	–	faecal egg count
FECRT	–	faecal egg count reduction test
GIN	–	gastrointestinal nematode
IQR	–	interquartile range
IVM	–	ivermectin
IVM-Ag	–	ivermectin aglycone
L1	–	first-stage larva
L2	–	second-stage larva
L3	–	third-stage larva
LC50	–	median lethal concentration 50%
LC55	–	lethal concentration 99%
LDT	–	larval development test
LEV	–	levamisole
MALDT	–	micro-agar larval development test
MDR	–	multidrug resistance
ML	–	macrocyclic lactone
MOP	–	monepantel
MOX	–	moxidectin
nAChR	–	nicotinic acetylcholine receptor
PCR	–	polymerase chain reaction
TBZ	–	thiabendazole
TST	–	targeted selective treatment
WAAVP	–	World Association for the Advancement of Veterinary Parasitology

INTRODUCTION

Sheep production is an important component of the livestock sector in many European countries, contributing to rural economies, landscape management, and agrobiodiversity [1]. However, the profitability and sustainability of sheep farming are substantially constrained by gastrointestinal nematodes (GIN), which remain among the most prevalent and impactful pathogens in grazing ruminants [2, 3]. Even when clinical signs are absent, GIN infections reduce weight gain, feed efficiency, and carcass quality and may impair reproductive performance, leading to considerable economic losses at the flock and sector level [4–6].

The most important GIN of sheep belongs to the order *Strongylida*, with *Teladorsagia circumcincta (T. circumcincta)*, *Trichostrongylus* spp., and *Haemonchus contortus* (*H. contortus*) recognised as the predominant and most pathogenic genera in temperate regions of Europe [3, 7]. Their epidemiology is shaped by interactions between parasite biology, climate, grazing management, and host immunity. Climate change – through rising temperatures and altered precipitation patterns – is expected to prolong the transmission season in northern Europe and favour highly pathogenic species such as *H. contortus* [8, 9]. These same genera are also most frequently implicated in anthelmintic resistance (AR), with nematode strains resistant to benzimidazoles (BZ), macrocyclic lactones (ML), and levamisole (LEV) repeatedly reported in European sheep flocks. Multidrug resistance (MDR) is now documented in at least ten countries, although its true distribution is probably wider than currently recognized [5, 6, 10].

As resistance to BZ, ML, and, increasingly, LEV has become widespread in small ruminant GIN, accurate detection and monitoring of AR have become essential for sustainable control [1, 5, 10]. *In vitro* assays are increasingly used to detect AR, with egg hatch tests (EHT) mainly applied for BZ resistance, while larval development tests (LDT), including micro-agar variants (MALDT), provide broader information on resistance to BZ, LEV, and ivermectin (IVM) but require more laboratory work. These methods offer high analytical sensitivity and can detect resistant subpopulations even when they form only a small fraction of the parasite population [11, 12]. In response to the growing impact of AR, international guidelines now emphasize integrated parasite management strategies that combine diagnostic monitoring with targeted and targeted-selective treatments (TST), maintenance of *refugia*, accurate dosing, improved pasture management, and appropriate quarantine measures to reduce selection pressure while safeguarding animal health and productivity [2, 13, 14].

Research on AR in small ruminants is often constrained by small, convenience-based samples, frequently drawn from self-selected farms or holdings near research institutions, which can distort prevalence estimates and limit external validity. In Europe, the median sample size for AR surveys is only five farms, and truly randomized studies remain scarce in the Nordic-Baltic region [5]. Norwegian data indicate that BZ resistance is lower in randomly selected flocks than in purposefully chosen ones, highlighting the strong influence of study design on reported resistance levels [15]. In Lithuania, previous *in vitro* and *in vivo* studies have been restricted to a few central and southern farms with a history of frequent anthelmintic use [16, 17].

Despite the increasing importance of AR in sheep production, data from Lithuania remain scarce. The limited number of previous studies and the lack of information on parasite control practices highlight the need for a comprehensive assessment of AR in Lithuanian sheep flocks and the factors associated with its development.

Aim of the study

To investigate the prevalence and level of anthelmintic resistance (AR) to gastrointestinal nematodes (GIN) in Lithuanian sheep flocks using the *in vitro* micro-agar larval development test (MALDT) and to identify farm management and treatment factors associated with the development of resistance.

Objectives of the study

1. To determine farm management and treatment practices associated with the emergence of AR in Lithuanian sheep farms based on farmer survey data.
2. To determine the prevalence and infection intensity of GIN in the investigated Lithuanian sheep flocks.
3. To determine the prevalence and level of AR to the main classes of anthelmintic drugs (IVM, BZ, LEV) using the MALDT, and to identify the main GIN genera associated with resistance.
4. To analyze the relationships between AR and sheep farm characteristics, as well as the classes of anthelmintics used on the farms.

Scientific novelty

The prevalence of sheep GIN resistance to BZ, IVM, and LEV was assessed using a systematic, population-based sampling approach-recruiting farms sequentially from the complete membership list of the Lithuanian Sheep Breeders Association without pre-selection based on suspected resistance. In contrast to previous Lithuanian AR studies [16, 17] which focused on a small number of non-randomly selected farms with intensive anthelmintic use, and consistent with recommendations for more rigorous epidemiological AR surveillance [5]. This work combines nationwide MALDT data with a cross-sectional questionnaire, providing the first systematic evidence on how specific treatment and management practices are associated with observed resistance patterns in Lithuanian sheep farms.

Practical significance

The study delivers representative estimates of resistance to BZ, IVM, and LEV in sheep nematodes and links these patterns to specific treatment and management practices. This baseline enables more rational use of anthelmintics, supports the adoption of integrated, risk-based parasite control strategies adapted to Lithuanian conditions, and offers practical recommendations to improve flock health, preserve drug efficacy, and enhance the sustainability of sheep production.

1. LITERATURE REVIEW

1.1. Main gastrointestinal nematode genera affecting sheep in Europe and the Baltic region

GIN infections of sheep in Europe are predominantly caused by nematodes belonging to the order *Strongylida* [2, 8, 18]. Among these, the genera *Teladorsagia* (formerly *Ostertagia*), *Trichostrongylus*, and *Haemonchus* are considered the most epidemiologically and economically important [2, 18]. These genera differ in their biological characteristics, pathogenicity, and responses to anthelmintic treatment, which have important implications for parasite control strategies and the development of AR [2, 19]. In temperate regions of Europe, *T. circumcincta* is regarded as the dominant abomasa nematode of sheep [2, 8]. This species is well adapted to cooler climates and exhibits seasonal transmission patterns closely linked to grazing management and environmental conditions [8, 9]. Infections with *T. circumcincta* are typically associated with reduced feed intake, impaired nutrient absorption, and decreased weight gain rather than acute clinical signs [2]. Due to its high prevalence and persistent exposure to anthelmintic treatments, *T. circumcincta* is frequently implicated in cases of AR, particularly to BZ and ML [19]. Nematodes of the genus *Trichostrongylus* spp. are widely distributed across Europe and are commonly found in both sheep and goats [8, 18]. These parasites primarily inhabit the small intestine and are often associated with diarrhoea, reduced growth performance, and poor body condition [8]. Although infections are usually subclinical, heavy burdens can result in significant production losses [18]. *Trichostrongylus* spp. are known for their ability to survive under a wide range of environmental conditions, which contributes to their persistence on pasture [8, 18]. *Trichostrongylus* spp. are frequently detected in mixed infections and are increasingly reported in association with AR [20]. *H. contortus* is considered one of the most pathogenic GIN of sheep due to its blood-feeding behaviour in the abomasum [18, 21]. Infection with this species can cause severe anemia, hypoproteinaemia, and sudden mortality, especially in lambs and young sheep [21]. Although traditionally associated with warmer climates, *H. contortus* has expanded its geographical range northwards in recent decades. Climate change, increased animal movement, and changes in grazing practices are believed to facilitate its establishment in regions previously considered unsuitable [9, 22]. In Europe, *H. contortus* is increasingly detected and represents a growing concern due to its strong association with multi-drug resistance (MDR) [2]. Recent parasitological surveys in the Baltic region confirm that *T. circum-*

cincta, *Trichostrongylus* spp. and *H. contortus* are also the predominant GIN genera in sheep farms, and they are frequently implicated in AR cases, including MDR [16, 23, 24].

1.1.1. Epidemiology and transmission of gastrointestinal nematodes in sheep farms

The epidemiology of GIN infections in sheep is determined by complex interactions between parasite biology, environmental conditions, host immunity, and farm management practices. Most economically important GIN species affecting sheep have a direct life cycle, in which eggs are excreted in faeces, develop into infective third-stage larvae (L3) on pasture, and are subsequently ingested by grazing animals [2, 8]. Environmental factors, particularly temperature and moisture, play a crucial role in the development, survival, and migration of infective larvae on pasture [8, 9]. In temperate regions of Europe, GIN transmission follows a seasonal pattern, with the highest infection pressure typically occurring from late spring to autumn. Climate-driven modelling studies indicate that rising temperatures and milder winters will prolong the transmission season of gastrointestinal nematodes, including *H. contortus*, and increase cumulative infection pressure in temperate and more northern parts of Europe compared with historical patterns. These effects are expected to be particularly relevant for northern and Baltic sheep-producing regions, where shorter grazing seasons have previously limited parasite development and overwinter survival [9, 22].

Host-related factors significantly influence GIN epidemiology. Lambs and yearlings are especially susceptible due to their immature immune systems and lack of previous exposure to parasites [2, 3]. In contrast, adult sheep generally develop partial immunity after repeated infections, which reduces worm fecundity and egg output but does not prevent reinfection. As a result, adult animals often act as asymptomatic carriers and continue to contaminate the pasture with parasite eggs, thereby sustaining infection pressure within the flock [3].

Farm management practices strongly affect GIN transmission dynamics. Grazing intensity, stocking density, and grazing system (continuous versus rotational grazing) influence pasture contamination and larval intake [8, 25]. Continuous grazing systems often result in higher infection pressure due to repeated exposure to contaminated pasture, whereas rotational grazing may reduce larval intake if adequate rest periods are applied [25]. However, poorly implemented rotational grazing can also increase infection risk if animals are returned to pasture before infective larvae have declined [8]. Recent research highlights substantial differences between farm heterogeneity in parasite burdens and species composition, even within the same geographic region, largely reflecting differences in management practices and anthelmintic use history [1]. Animal movement between farms represents an increasingly important pathway for the spread of GIN infections and AR parasite populations. The introduction of infected animals without appropriate quarantine and diagnostic testing can rapidly alter the resistance profile of a flock [26, 27]. Studies across Europe have demonstrated that livestock trade and insufficient biosecurity contribute significantly to the dissemination of resistant nematode strains between regions and countries [5, 28–30]. Even the introduction of a small number of resistant parasites can have long-term consequences, particularly in production systems characterized by frequent anthelmintic treatments and limited *refugia*, which strongly favour the selection and persistence of resistant genotypes [31–33].

Overall, the epidemiology of GIN in sheep farms is multifactorial and highly dynamic. Effective control, therefore, requires an integrated understanding of parasite life cycles, environmental drivers of transmission, host immunity, and management-related risk factors. Such knowledge is essential for the development of sustainable parasite control strategies aimed at reducing infection pressure while minimizing the selection for AR.

1.2. Anthelmintic groups used in sheep production

1.2.1. General overview

Anthelmintics remain the primary tool for controlling GIN in sheep production systems worldwide. Despite advances in diagnostics and management strategies, chemical control continues to underpin parasite management due to its rapid efficacy and ease of application. However, the intensive and often prophylactic use of anthelmintics has led to widespread AR, now recognized as one of the most significant constraints on sustainable sheep production globally [2, 34]. Data from Europe indicate that resistance has been reported against all major anthelmintic classes used in sheep, with particularly high prevalence for BZ and ML [5].

1.2.2. Benzimidazoles

BZ, including albendazole, fenbendazole, and thiabendazole, were the first broad-spectrum anthelmintics introduced for nematode control in sheep. Their mode of action involves selective binding to β-tubulin, disrupting microtubule polymerization and inhibiting essential cellular processes in nematodes [35, 36]. Resistance to BZ emerged rapidly following its widespread adoption and is now the most prevalent form of AR in small ruminants globally [19]. Molecular studies have identified point mutations in the β-tubulin gene particularly at codons 167, 198, and 200, as the primary mechanism underlying resistance [37]. European surveys consistently report BZ resistance in *H. contortus*, *T. circumcincta*, and *Trichostrongylus* spp., often exceeding 80% of investigated farms [10, 38]. In Lithuania, BZ resistance has been detected across multiple regions using EHT and MALDT [16]. Given its broad spectrum, low cost, and long-standing use, BZ continues to play a central role in parasite control on many sheep farms, but its compromised efficacy in numerous regions has substantially reduced the available options for sustainable GIN management [10, 19].

1.2.3. Imidazothiazoles (levamisole)

LEV, the primary representative of the imidazothiazole class used in sheep, acts as a nicotinic acetylcholine receptor agonist, inducing spastic paralysis of nematodes and rapid worm expulsion, but with a relatively short persistence compared with ML. Historically, LEV resistance developed more slowly than resistance to BZ and ML, contributing to its continued use as a strategic alternative, particularly in combination products or rotation schemes [34]. Nevertheless, resistance to LEV has been increasingly reported in several European countries, particularly in flocks exhibiting MDR [39, 40]. *In vitro* LDT has demonstrated high sensitivity for detecting LEV resistance, even at low resistance allele frequencies [41]. In contrast to trends in Western and Southern Europe, studies from northern Europe, particularly the Nordic countries, indicate that LEV remains largely effective, with no resistance detected in nationwide surveys, possibly reflecting lower historical usage intensity and a stronger reliance on BZ and ML in past decades [15, 41]. In practice, the relatively narrow safety margin of LEV and the need for accurate dosing have been cited as reasons for its more cautious use on farms, which may have contributed to preserving its efficacy in some regions [19, 34]. Consequently, LEV is increasingly recommended as a key component of integrated parasite management strategies, particularly on farms where BZ and ML efficacy is compromised [42].

1.2.4. Macrocyclic lactones

ML, including IVM, moxidectin (MOX), doramectin, and abamectin, are among the most widely used anthelmintics in sheep production. These compounds act by binding to glutamate-gated chloride channels, causing neuromuscular paralysis and death of nematodes [34]. Resistance to MLs was initially considered slow to develop; however, widespread resistance is now reported globally. European meta-analyses show that ML resistance prevalence exceeds 50%, with ivermectin resistance the most common [5]. ML resistance has been documented extensively in *H. contortus* and *T. circumcincta*, including cases of treatment failure with moxidectin, previously considered a "last-resort" compound [43, 44]. Although MOX has traditionally been regarded as a "last-resort" anthelmintic due to its higher lipophilicity, prolonged persistence in host tissues, and enhanced efficacy against ivermectin-resistant nematode populations, increasing evidence from several European regions challenges this assumption. Documented treatment failures and reduced efficacy of MOX against *T. circumcincta* and *H. contortus* have been reported in the United Kingdom and other European countries, particularly in flocks with a history of intensive ML use [43, 45]. These findings are of particular concern because resistance to MOX often arises in the context of pre-existing IVM resistance, suggesting partial cross-resistance within the macrocyclic lactone class rather than independent resistance mechanisms [34, 36]. Moreover, due to the limited number of alternative anthelmintic classes currently available for sheep, the erosion of MOX efficacy substantially narrows future control options and threatens the long-term sustainability of chemical parasite control strategies in sheep production systems [5, 46].

1.2.5. Amino-acetonitrile derivatives and other novel anthelmintics

Amino-acetonitrile derivatives, represented by monepantel (MOP), were introduced as a novel anthelmintic class with a distinct mode of action targeting nematode-specific acetylcholine receptors. Initially highly effective against MDR nematode populations, MOP resistance has now been reported in multiple countries, including Australia, the United Kingdom, Sweden, and Belgium [45, 47–49]. Of particular concern are reports of MDR to MOP, which significantly compromise its utility as a resistance-breaking compound [44, 48]. Although MOP retains good efficacy on many farms, current evidence suggests that, once resistance has emerged, reversion to full susceptibility is unlikely under field conditions, underscoring the need for cautious, targeted use rather than routine prophylaxis [47].

1.2.6. Implications for sustainable parasite control

The widespread occurrence of resistance across all major anthelmintic classes used in sheep production has been widely documented and demonstrates the limitations of relying solely on chemical control strategies. Consequently, increasing attention has been directed towards integrated parasite management approaches aimed at reducing selection pressure on nematode populations. Key elements of such approaches include targeted selective treatment, maintenance of parasite *refugia*, accurate dosing, and the application of quarantine measures, all of which have been shown to contribute to slower resistance development [46, 50]. Recent studies further indicate that routine prophylactic treatments, limited use of diagnostic tools, and frequent underdosing remain common practices in sheep production systems and represent important drivers of anthelmintic resistance [2, 42].

1.3. Anthelmintic resistance: definition, mechanisms of emergence and risk factors

1.3.1. Definition of anthelmintic resistance

AR is defined as the heritable ability of a parasite population to survive doses of an anthelmintic that are normally effective against the same species and developmental stage. This definition, formalized by the World Association for the Advancement of Veterinary Parasitology (WAAVP), highlights resistance as a genetically determined, population-level phenomenon rather than an individual treatment failure [51]. Resistance emerges through natural selection acting on pre-existing genetic variation within parasite populations following repeated exposure to anthelmintic compounds. As susceptible individuals are removed, resistant parasites survive and reproduce, resulting in a progressive increase in the frequency of resistance-associated alleles over successive generations [2, 34]. Numerous studies indicate that reversion to susceptibility following withdrawal of drug pressure is uncommon and, where reported, tends to be transient under field conditions [34, 46]. In sheep GIN, AR has been documented against all major anthelmintic classes, including BZ, LEV, ML, and, more recently, amino-acetonitrile derivatives [5, 52].

1.3.2. Mechanisms of emergence of anthelmintic resistance

AR in GIN results from specific molecular adaptations in parasites, combined with strong selection pressure from anthelmintic use. While AR is expressed at the population level, it is underpinned by distinct mechanisms that reduce drug susceptibility and by management practices that favor the survival and spread of resistant parasites [53–55]. For BZ, which binds to β-tubulin, target-site alterations are the best-characterized mechanism of resistance. Non-synonymous single nucleotide polymorphisms at codons 167, 198 and 200 (F167Y, E198A, F200Y) in the isotype-1 β-tubulin gene reduce BZ binding and have been consistently associated with resistance in *H. contortus*, *T. circumcincta*, and *Trichostrongylus* spp. Systematic and genomic studies indicate that these mutations can rapidly reach high frequencies under intensive BZ use and are now widespread in small ruminant nematode populations globally [54, 56–58]. Resistance to ML is generally more complex and polygenic. MLs primarily target glutamate-gated chloride channels and other ligand-gated ion channels, and reduced susceptibility has been linked to changes in channel subunit genes, altered receptor expression, and upregulation of ATP-binding cassette transporters, particularly P-glycoproteins. Recent genomic and transcriptomic work in *H. contortus* and other strongylids supports a role for multiple loci affecting drug uptake, neuronal signalling and detoxification pathways, which together modulate ML sensitivity [53, 54, 56, 57, 59, 60]. For imidazothiazoles and tetrahydropyrimidines, resistance is mainly associated with modifications in nicotinic acetylcholine receptors (nAChRs). Reduced expression of genes encoding specific nAChR subunits and changes in receptor composition can markedly decrease drug sensitivity while preserving endogenous cholinergic function and point mutations in nAChR genes have been described in resistant nematode and model *Caenorhabditis elegans* strains. Similar target-site loss-of-function phenomena have also been implicated in resistance to newer nicotinic agonists such as monepantel [53, 54, 56, 59]. In addition to target-site alterations, non-target mechanisms such as upregulation of detoxification enzymes and efflux transporters can reduce effective drug exposure at parasite target sites and contribute to multi-class resistance [53, 56]. Taken together, these molecular adaptations provide the biological basis for anthelmintic resistance, while the rate at which they emerge and spread in the field is further shaped by farm-level selection pressure and the size of parasite *in refugia* [2, 61].

1.3.3. Risk factors associated with the development of anthelmintic resistance

The development of AR in GIN is strongly influenced by how anthelmintics are used at the farm level and by management practices that modulate selection pressure and *refugia* size. Key risk factors include treatment frequency and timing, dosing accuracy, drug choice and rotation, quarantine procedures for purchased animals, and grazing management strategies [46, 55, 62, 63]. One of the most important drivers of AR is frequent, whole-flock treatment, especially when administered routinely on a calendar basis without parasitological diagnostics. Mass treatments expose a large proportion of the parasite population to anthelmintics and markedly reduce the contribution of worms *in refugia*, thereby accelerating the increase in resistance allele frequencies. The use of long-acting formulations and prophylactic treatments at fixed times (e.g., before turnout or housing) further intensifies selection pressure and has been repeatedly associated with the rapid development of resistance in sheep flocks [46, 55, 64–66].

Inaccurate dosing represents another major risk factor. Underdosing can arise from reliance on visual estimation of body weight, failure to base the dose on the heaviest animals in the group, poorly calibrated drenching equipment, or improper storage and handling of anthelmintic products. Subtherapeutic drug concentrations are particularly problematic because they allow survival of partially resistant worms and heterozygotes, thereby accelerating the selection and stabilization of resistance within parasite populations. Field and modelling studies consistently indicate that underdosing can have a stronger impact on AR development than treatment frequency alone [46, 55, 63, 65]. Drug choice, rotation patterns, and the use of combination treatments also affect resistance risk. Prolonged reliance on a single anthelmintic class, especially BZ or ML, has been linked to high levels of class-specific resistance in many sheep-producing regions. Conversely, inappropriate or unsystematic rotation between classes may fail to reduce selection pressure if changes are introduced only after resistance is established. Combination treatments can help delay resistance when both components remain highly effective, but their use on farms where one or more classes already show reduced efficacy may instead promote MDR by simultaneously selecting for parasites carrying multiple resistance mechanisms [55, 65, 66].

Quarantine and animal movement practices represent additional critical risk factors. Introduction of untreated, infected animals from other farms or countries can rapidly disseminate resistant nematode strains into previously susceptible flocks. Failure to implement effective quarantine protocols, including isolation, appropriate anthelmintic treatment with follow-up diagnostics, and controlled turnout onto contaminated pastures, greatly increases the likelihood that imported resistant worms will establish and spread. Several studies have highlighted livestock trade and inadequate biosecurity as major drivers of the regional and international spread of AR [64, 65, 67].

Grazing management and *refugia*-based strategies play a central role in modulating selection for resistance. Practices such as "dose-and-move" in which animals are treated and immediately moved to low-contamination or "clean" pastures, drastically reduce the proportion of susceptible parasites in *refugia* and favour the rapid expansion of resistant genotypes. In contrast, approaches that deliberately maintain *refugia* – such as leaving a proportion of animals untreated or delaying movement to low-contamination pastures after treatment have been proposed to slow AR development, although implementation and evidence from field trials remain challenging. Overall, the combination of intensive drug use, inadequate dosing and biosecurity, and grazing strategies that minimize *refugia* creates a high-risk environment for the emergence and spread of anthelmintic resistance in sheep nematodes [46, 55, 63, 64, 66].

1.4. Diagnostic methods for detecting anthelmintic resistance

Reliable diagnosis of AR is essential for monitoring its prevalence and for adapting control strategies in sheep production systems [2, 68, 69]. Phenotypic methods assess parasites' response to treatment, whereas molecular methods detect genetic markers associated with resistance. Each group of methods has distinct characteristics in terms of sensitivity, specificity, and practicality under field conditions [2, 53, 68].

1.4.1. *In vivo* methods

The faecal egg count reduction test (FECRT) remains the reference *in vivo* method for assessing anthelmintic efficacy under field conditions [51, 68]. In FECRT, faecal egg counts (FEC) are measured before and after treatment, usually 10–14 days post-treatment, depending on the drug, and the percentage reduction in egg output is used to infer efficacy and the presence of resistance [51, 70]. Recently updated WAAVP guidelines provide detailed recommendations for FECRT design, sampling, faecal egg counting techniques, and statistical analysis to improve standardisation and diagnostic performance [20, 71]. However, FECRT sensitivity is limited when resistance alleles are still at low frequencies or when egg counts are low and highly aggregated, and results are influenced by host factors and methodological variability [68, 71, 72]. In addition, FECRT cannot readily distinguish which nematode genera or species are resistant in mixed infections [69, 73]. Besides FECRT, controlled *in vivo* efficacy tests based on post-mortem worm counts are regarded as the gold standard for assessing anthelmintic resistance, because they directly quantify reductions in worm burdens in treated animals compared with untreated controls. Assessing all five licensed anthelmintic groups, including IVM and MOX, along with a control group, requires a substantial number of animals and strict experimental conditions, which further limits the feasibility of these tests on commercial farms. As a result, most field studies evaluate only a limited range of anthelmintics. While the controlled *in vivo* efficacy test is the gold standard for assessing AR, its reliance on animal slaughter makes it unsuitable for routine monitoring in commercial settings [5, 6].

1.4.2. *In vitro* assays

In vitro assays have been developed to complement FECRT and to increase the analytical sensitivity of AR detection [68, 73, 74]. Various *in vitro* tests are available to detect AR, with the EHT being the most, particularly for BZ resistance [51]. However, the LDT, including MALDT variants, is considered the most efficient for detecting resistance to BZ, LEV, and IVM, despite being more laborious and time-consuming [12, 50, 51]. LDT is applicable for detecting resistance to all broad-spectrum anthelmintics, including ML, whereas EHT is essentially limited to BZ resistance detection [75, 76]. *In vitro* tests have also demonstrated high analytical sensitivity, with LDT / MALDT able to identify resistant parasites even when they comprise as little as 4% of the [12, 76]. Nevertheless, these assays require standardized laboratory conditions and expertise in larval identification and are therefore less practical for routine on-farm screening [73, 74].

1.4.3. Molecular methods

Molecular diagnostic methods target specific genetic changes associated with AR and offer the potential for highly sensitive and rapid detection [14, 74]. For BZ resistance, numerous polymerase chain reaction (PCR)-based assays have been developed to detect single-nucleotide polymorphisms in the β-tubulin isotype-1 gene, particularly at codons 167, 198, and 200, which are strongly associated with reduced BZ susceptibility in *H. contortus* and other trichostrongylid nematodes [53, 77]. Quantitative real-time PCR and droplet digital PCR enable accurate estimation of resistance allele frequencies in field samples and allow detection of resistant genotypes even at very low proportions [78, 79]. Other candidate molecular markers, such as changes in nicotinic acetylcholine receptor subunit genes for levamisole resistance and increased expression of P-glycoproteins and other transporters for macrocyclic lactone resistance, are also being investigated, although their robustness across diverse parasite populations is not yet fully established [14, 53]. Despite their high analytical sensitivity, molecular methods currently detect only known resistance markers and may not capture all mechanisms or newly emerging mutations, particularly in cases of polygenic resistance [14, 53]. For this reason, molecular diagnostics are best viewed as a complement rather than a replacement for phenotypic assays, especially when the clinical significance of the detected alleles needs to be interpreted in field conditions [53, 74]. Additionally, molecular tests remain limited to detecting BZ resistance [19].

1.4.4. Comparison of diagnostic methods in terms of sensitivity and specificity

Comparative evaluations demonstrate that no single diagnostic test provides optimal sensitivity and specificity for all anthelmintic classes and epidemiological settings [68, 69, 74]. FECRT is highly valuable as a whole-flock field tool, but its sensitivity decreases when resistance is at an early stage and is strongly influenced by study design, egg counting methods, and statistical analysis [71, 72]. *In vitro* assays such as EHT and LDT / MALDT generally show greater analytical sensitivity because they directly measure parasite development at defined discriminating concentrations and can detect resistant subpopulations that remain undetected by FECRT [68, 76, 80]. Molecular methods often achieve very high analytical sensitivity and specificity for markers, for example, β-tubulin polymorphisms, but their scope is restricted to the mutations they are designed to detect, and they do not directly quantify phenotypic resistance at the population level [77–79]. Consequently, recent reviews and guidelines advocate integrated diagnostic approaches that combine FECRT with selected *in vitro* tests and molecular assays, tailored to local parasite species and drug-use patterns, to improve the accuracy and timeliness of AR detection in ruminant nematodes [14, 53].

1.5. Anthelmintic resistance in sheep gastrointestinal nematodes in Europe

Over the last four decades, AR in sheep and goat GIN has become widely established across Europe and continues to increase, although important knowledge gaps remain in some areas, particularly in eastern Europe [5]. Recent European reviews emphasize that AR should now be regarded as endemic in many small-ruminant systems, and that without substantial changes in worm control practices, further erosion of drug efficacy is likely [1]. In most European countries, recent AR reports predominantly describe reduced efficacy of BZ, alongside a growing number of cases involving ML, especially IVM [5, 10]. According to the meta-analysis by Vineer *et al.* [5], which included studies on sheep and goats in Europe published since 2010, the average farm-level prevalence of resistance was 86% for BZ, 52% for ML, 48% for LEV, and 21% for MOX, when single-farm case reports were excluded. These figures indicate that, for many farms, at least one major anthelmintic class can no longer be relied upon for effective control, and that combinations of resistance are increasingly common rather than exceptional outcomes [1]. Additional reports describe resistance to doramectin in the Netherlands and to MOX in Switzerland and southern Germany [30, 81], as well as to MOP in the United Kingdom [82]. Multiple AR in sheep GIN has been reported in France, Greece, Italy, the United Kingdom, and Scotland [39, 83–85]. Across Europe, BZ and IVM remain the most commonly used anthelmintics in small ruminants, yet their efficacy is severely compromised in several countries: BZ resistance has been detected on up to 98% of examined sheep farms in the Czech Republic [86], 14% in Spain [87], 100% in the Slovak Republic [80] and 100% in Ireland [88]. In the UK, BZ resistance occurs on 98% of farms, and on 64% of farms in Scotland alone; in many regions, IVM and LEV are also considered insufficiently effective [39, 82, 89, 90]. In Belgium, sheep farms have shown resistance to BZ, ML, closantel, and MOP [49]. Furthermore, studies from Poland have demonstrated that BZ and IVM resistance are present on 89% and 76% of sheep farms, respectively, confirming that AR is also highly prevalent in Central and Eastern Europe [91, 92]. Taken together, these reports from Western, Central, and Eastern Europe demonstrate that AR is no longer restricted to isolated farms or specific regions but represents a continent-wide challenge for sustainable small-ruminant production [1].

AR has become a significant concern across the Nordic region, with studies from Denmark, Sweden, Norway, and Lithuania documenting its spread. The earliest evidence of resistance in sheep nematodes in this area was reported in Denmark in 1991, describing LEV resistance in *Teladorsagia* [93]. Subsequent Danish studies identified resistance to BZ, IVM, and LEV in *trichostrongyle* nematodes in goats and sheep [94, 95]. Another Danish case revealed concurrent resistance to BZ and IVM, although MOX and LEV remained effective [96]. In Sweden, AR levels were relatively low. Studies from the 1990s implicated BZ resistance in *H. contortus*, with IVM still highly effective [97]. A later survey found albendazole resistance in only 4% of 45 farms investigated, though genetic testing showed wider distribution of the β-tubulin codon 200 mutation [98]. Recent findings indicate the emergence of IVM resistance in *H. contortus* among Swedish sheep flocks, likely driven by the introduction of resistant strains through the importation of animals. A case study on a few farms, including one on Gotland that had imported sheep from the Netherlands, confirmed this development [99]. Multi-resistance to BZ and IVM has now been reported on several Swedish farms, and one case even involved triple resistance, including monepantel (MOP), leaving LEV as the only effective option on some farms [48]. In Norway, nationwide monitoring indicated a relatively low BZ resistance level (11%) among sheep flocks, but regional differences exist. In Rogaland County, 80% of intensively treated or frequently dewormed flocks showed BZ resistance [15]. A recent case also confirmed IVM resistance in sheep, while AR to BZ has so far been identified in only one goat herd [100]. In

Lithuania, several surveys conducted in 2013–2014 revealed BZ resistance in 20% and IVM resistance in 13% of tested sheep farms, based on FECRT [24]. *In vitro* studies demonstrated broader resistance patterns: using EHT, BZ-resistant GIN were detected on all 25 farms, and by MALDT, on 71% of farms. IVM resistance was found on 61.9%, and LEV resistance on 33% of the tested farms [16, 24].

1.5.1. Multidrug resistance in the Nordic-Baltic region

MDR may ultimately lead to complete anthelmintic failure and is considered one of the greatest challenges to sustainable nematode control [28]. In the Nordic countries, MDR has become an emerging concern. Veterinary data from the Swedish Farm and Animal Health database show a growing number of farms with treatment failures to both IVM and BZ, and reduced efficacy of MOP has also been observed in at least one flock [84]. In Lithuania, FECRT identified combined resistance to BZ and ML on one farm, while *in vitro* assays demonstrated MDR to both drug classes on five of 13 farms (38.5%) [17]. In Denmark, FECRT confirmed multiple resistance to IVM and BZ in seven goat herds (70%) [101] and *Trichostrongylus colubriformis* isolates resistant to both drugs were reported [98]. Dual resistance to IVM and BZ has also recently been documented in a Norwegian sheep flock [100]. Overall, available evidence indicates that AR, including MDR, is now widespread in Europe. However, many existing studies are based on convenience sampling or isolated case reports, and robust prevalence data from randomized farm surveys remain limited. Recent European reviews underline that effective and sustainable GIN control will require a shift from routine prophylactic treatments toward integrated parasite management and targeted selective treatment strategies [1].

1.6. Nematode genera involved in AR in the Nordic-Baltic region

In Norwegian dairy goat herds, pre-treatment GIN burdens were generally low compared with those observed in sheep flocks, and AR to BZ in *Teladorsagia* and *Trichostrongylus* was confirmed in only a single herd [15]. In Lithuania, studies employing various AR detection methods similarly identified *Teladorsagia* and *Trichostrongylus* as the predominant genera associated with resistance [17, 24]. In contrast, Swedish data suggest that AR is predominantly associated with *H. contortus*, although other genera, including *Teladorsagia* and *Trichostrongylus*, may also contribute according to droplet digital PCR results [102]. A Swedish nematobiome study further demonstrated that different nematode species varied in their responses to

IVM, BZ, and LEV. Of relevance, both IVM and BZ failed to adequately control *H. contortus* on several farms, whereas LEV retained comparatively good efficacy; nevertheless, *H. contortus* frequently persisted at low levels even after LEV treatment. *T. circumcincta* also survived treatment with either IVM or BZ, although to a lesser extent than *H. contortus*, and was not detected following LEV administration [103]. In Denmark, a controlled efficacy test confirmed AR in *H. contortus* and *Trichostrongylus colubriformis* isolates originating from a mixed sheep and goat farm [96].

1.7. Anthelmintics and treatment practices in the Nordic-Baltic region

Several questionnaire-based studies have examined parasite control practices in small ruminant farms across northern Europe. In Denmark, a 1993 survey found that nearly all sheep farmers (97%) routinely used anthelmintics, averaging two treatments annually, with BZ as the predominant drug class, while IVM had just entered the market [94]. Later research on Danish goat farms showed similar trends: 89% used anthelmintics, typically once per year, with IVM being most common (63%) [101]. In Norway, surveys in 2007 revealed that most farmers estimated animal weight visually for dosing (79–85%) and that BZ was the main anthelmintic used in sheep (65%), while goats received BZ and ML in similar proportions. Almost half of farmers never changed the drug class, and one-third used dose-and-move strategies [104, 105]. A Swedish survey involving 39 commercial sheep farms reported only slight differences between conventional and organic systems. Nearly all farms (97%) used anthelmintics, mainly IVM and BZ, treating animals once or twice per year. Most practiced pasture rotation and occasionally confirmed treatment efficacy, while some still applied blanket treatments without diagnostics [106]. In Lithuania, surveys conducted in 2015–2016 among 71 sheep and 37 goat farms showed that anthelmintic use remained widespread (72% and 54%, respectively). IVM and BZ were the dominant drug classes, while LEV was rarely applied. Sheep were usually treated twice per year, goats once, and dosing rarely differentiated between age groups [17, 107].

2. MATERIALS AND METHODS

The study was carried out at the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, Veterinary Academy, Lithuanian University of Health Sciences, from 2018–2025. The research was carried out in accordance with the Lithuanian animal welfare regulations (No. B1-866, 2012; No. XI2271, 2012) and was approved by the Lithuanian Committee of Veterinary Medicine and Zootechnical Sciences (Protocol No. 07/2010).

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2.1. Design of the study

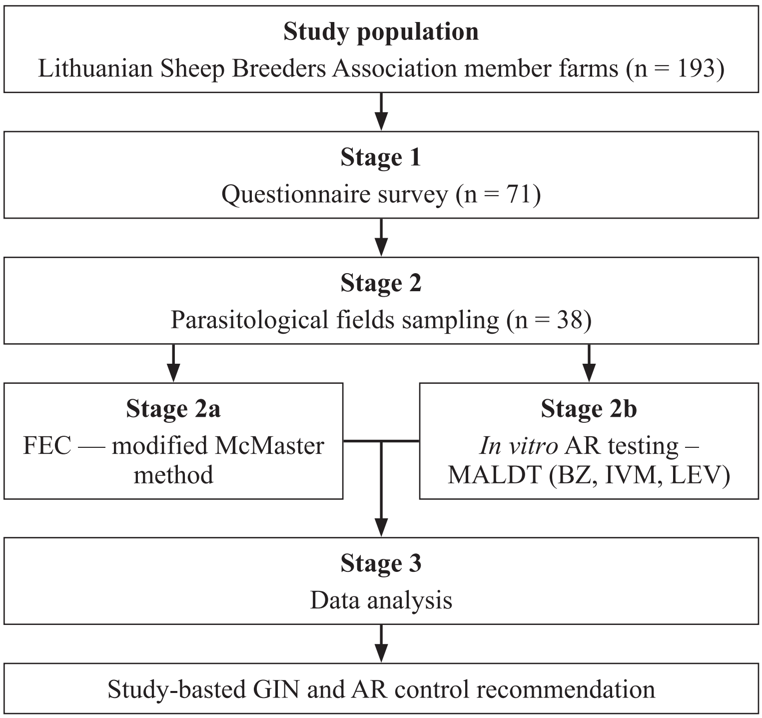


Fig. 2.1.1. *Study design*

2.2. Questionnaire survey

A cross-sectional questionnaire survey was employed to gather information on sheep farming practices among members of the Lithuanian Sheep Breeders Association. The survey was conducted during 2022–2023 and targeted all 193 members of the association, which represents sheep farms across Lithuania. Data collection was conducted via telephone interviews, directly conducted by the authors. In total, 71 respondents completed the questionnaire, resulting in a response rate of 36.8%. Of the remaining members, 73 were not reached despite repeated call attempts, while 49 declined to participate. Prior to the interviews', verbal informed consent was obtained, and subsequently, a written consent form was sent to all participants for documentation.

The questionnaire was adapted and modified from a survey instrument developed within the COST Action COMBAR Modifications were made to reflect the objectives of the present study and the specific conditions of sheep farming in Lithuania. The final questionnaire consisted of 16 items, including nine multiple-choice and seven open-ended questions. The instrument was structured to obtain detailed information on factors relevant to parasite control in sheep farms. The thematic areas covered were: (1) general farm characteristics (4 items); (2) grazing management (3 items), addressing grazing duration and system; and (3) parasite control strategies (9 items), covering treatment schedules, frequency and dosage of anthelmintics used, as well as quarantine practices for newly introduced animals (Accessory No 1).

2.3. Selection of farms for the anthelmintic resistance study

Sheep farms were recruited sequentially from the complete membership list of the Lithuanian Sheep Breeders Association, which comprised 193 registered farms at the time of the study. All farmers were contacted by telephone in list order and invited to participate. The only inclusion criterion was that farms must have at least 15 sheep to ensure a representative sample of actively managed flocks with established anthelmintic treatment practices. Before sampling, farmers were asked whether anthelmintic treatment had been administered within the previous 8 weeks. This information was collected to minimize the potential influence of recent treatment on faecal egg counts and *in vitro* resistance testing. A total of 38 sheep farms that agreed to participate and met the inclusion criterion were enrolled in the study, representing 19.7% of all invited farms. The participating farms were located across all ten Lithuanian counties, ensuring broad geographical coverage of the national sheep farming sector. The sample size was determined based on practical feasibility and insights from similar studies [24, 80]. Sequential recruitment from the complete membership list of the Lithuanian Sheep Breeders Association, without pre-selection based on suspected resistance, was chosen to minimise sampling bias, as non-random sampling has been shown to substantially overestimate AR prevalence [5, 15].

2.4. Modified McMaster method

FEC was determined using a modified McMaster technique with an analytical sensitivity of 20 EPG. Sampling was conducted during the grazing season. Within each flock, animals were selected using convenience sampling, with 10–15 animals per flock. To obtain a representative flock-level estimate of gastrointestinal nematode infection, all sheep older than one year of age were eligible for inclusion, irrespective of their age category. Individual faecal samples were collected either directly from the rectum or from freshly deposited faeces on the ground (not exceeding one hour old). All samples were stored and analyzed within 48 hours of collection using the modified McMaster method described above. For each examination, 4 g of faeces were weighed and thoroughly homogenized in 56 mL of tap water, and the resulting suspension was left to stand for approximately 30 min to allow large particles to soften. The faecal suspension was then poured through a tea strainer or a double layer of cheesecloth into a plastic beaker to remove coarse debris. From the filtered suspension, 10 mL were transferred into a centrifuge tube and centrifuged for 7 min at 1200 rpm. After centrifugation, the supernatant was discarded, and the remaining sediment was resuspended in a zinc chloride flotation solution (specific gravity 1.40) up to the 4 mL mark. The sediment–flotation mixture was carefully mixed, and both chambers of a McMaster counting slide were immediately filled with the suspension, avoiding air bubbles. After allowing the chambers to stand for approximately 3 minutes to enable the eggs to float to the surface, all strongylids (*T. circumcincta*, *Trichostrongylus* spp., *H. contortus*, *Cooperia* spp.), *Nematodirus* spp., *Strongyloides* spp. and *Trichuris* spp. eggs within the grids of both chambers were counted under a light microscope. Faecal egg counts (FEC) were calculated by summing the egg counts from both chambers and multiplying the total by 20.

2.5. *In vitro* anthelmintic resistance testing

2.5.1. Eggs preparation for *in vitro* method

Faecal samples were collected as described in section 2.4, from the same 38 sheep flocks that participated in the FEC survey, with 6–10 g collected per animal, yielding a pooled sample of approximately 100–120 g. The pooled material was homogenized, and one-third was transferred into a 250 mL container, which was then filled to two-thirds with tap water and sealed until further processing. Nematode eggs were isolated using a sequential sieving method. The faecal suspension was passed through sieves with mesh sizes of 250, 100, and 20 μm. The material retained on the 20 μm sieve was rinsed into a glass vessel, allowed to sediment for 15–20 minutes, and the supernatant was carefully removed. The remaining sediment was transferred into centrifuge tubes and centrifuged for 2 minutes at 1500 rpm. The supernatant was discarded, and saturated sugar solution was added until a meniscus formed, followed by a second centrifugation step. Eggs adhering to the coverslips were rinsed with distilled water into a clean vessel. The resulting egg suspension was washed with deionized water to remove sugar residues. Aliquots of the suspension were then taken using a micropipette, and egg counts were performed. The suspension was adjusted to a concentration of approximately 100 eggs per 10 μL to ensure standardization of subsequent analyses [24, 80].

2.5.2. Preparation of yeast extract for the *in vitro* test

A yeast extract solution was prepared by dissolving 1 g of yeast extract in 90 mL of 0.85% NaCl solution. The suspension was sterilized by autoclaving at 121 °C for 20 minutes. After autoclaving, 3 mL of concentrated Earle's balanced salt solution were added to 27 mL of the yeast extract solution. The prepared mixture was aliquoted and stored frozen for several months until use [108]. The composition of the 10× Earle's solution (g/100 mL) was as follows: KCl 0.04, $MgSO_4$ 0.2, NaCl 6.68, $NaHCO_3$ 2.2, NaH_2PO_4 0.14, and $CaCl_2$ 0.02.

2.5.3. Micro-agar larval development test

MALDT was performed according to the protocol described by Coles et al. [19]. Assays were conducted in 96-well microtiter plates. Stock solutions of ivermectin aglycone (IVM-Ag) and thiabendazole (TBZ) were prepared by two-fold serial dilution in dimethyl sulfoxide (DMSO), whereas LEV was diluted in deionized water. TBZ was used as the representative compound for BZ class resistance testing, while IVM-Ag served as the representative compound for IVM resistance testing. Twelve concentrations were tested: 0.084–173.6 ng/mL for IVM-Ag, 0.0006–1.28 µg/mL for TBZ, and 0.0156–32 µg/mL for LEV. For each concentration, 12 µl of the drug solution was added to 150 µL of 2% Bacto agar (Difco, USA), and the mixture was allowed to solidify at 4 °C for 5 minutes. To prevent fungal contamination, 10 µL of egg suspension (approximately 50 eggs per well) in 0.3 mg/mL amphotericin B (Sigma-Aldrich, Germany) was combined with 10 µL of yeast extract and layered onto the agar. The yeast extract was prepared following the method of Hubert & Kerboeuf [108]: 1 g of yeast extract dissolved in 90 mL of 0.85% NaCl, autoclaved for 20 min, and then supplemented with 3 mL of 10× concentrated Earle's solution per 27 mL of stock. Control wells contained 1.3% DMSO. Plates were incubated for 7 days at 27 °C. Development was terminated by adding Lugol's iodine solution to each well. The proportion of unhatched eggs and larvae at L1, L2, and L3 stages was assessed using an inverted stereomicroscope [16, 24].

The MALDT data were analysed using the discriminant concentration method. Farms were classified as resistant when L3 larvae were detected at the following discriminating concentrations: 21.6 ng/mL for IVM-Ag, 0.04 µg/mL for TBZ, and 2 µg/mL for LEV [16, 24, 76, 80, 109]. L3 at discriminating concentrations were differentiated and identified based on morphological features [50].

2.6. Statistical and data analysis

2.6.1. General statistical procedures

Data analysis was performed using Microsoft Excel 2019 and IBM SPSS Statistics, version 29.0 (IBM Corp., Armonk, NY, USA; 2022). Descriptive statistics were used to summarize the dataset and characterize farm-level variables. Continuous variables were summarized using means, medians, interquartile ranges, and ranges. Categorical variables were expressed as proportions with 95% confidence intervals (CI) to indicate the precision of the estimates. Confidence intervals for proportions were calculated using the Clopper–Pearson method based on a one-sample binomial distribution, while Wilson score intervals were used for comparisons of binomial proportions.

Associations between categorical variables were evaluated using Pearson's Chi-square test. When the expected cell count was less than five, Fisher's Exact Test was employed to ensure the robustness of the statistical inference. This approach was also applied to analyses of anthelmintic resistance (including multidrug resistance, MDR), where resistance status (resistant *vs* susceptible) was cross-tabulated with farm-level factors such as breed class, use of specific anthelmintics, and presence of imported sheep. Comparisons of continuous variables, including average treatment frequency, between traditional and organic farms were conducted using either independent samples t-tests or Mann-Whitney U tests, depending on the data's distribution. The threshold for statistical significance was set at $p < 0.05$.

Stocking density (sheep per hectare) was calculated by dividing the total number of sheep by the reported pasture area. It was analyzed as both a continuous and a categorical variable and classified into three categories: low (< 3 sheep/ha), moderate (3–7 sheep/ha), and high (> 7 sheep/ha).

2.6.2. Analysis of FEC and *in vitro* test results

The proportion of developed L3 larvae at these discriminating concentrations was used to categorize the degree of resistance as mild (< 10%), moderate (10–50%), or severe (> 50%) [20]. This classification reflects the proportion of larvae that survived and completed development despite exposure to a drug concentration lethal to susceptible populations – a higher proportion of surviving L3 indicating a greater frequency of resistant individuals within the nematode population. This approach therefore enabled not only binary detection of resistance (present *vs* absent) but also quantitative evaluation of resistance intensity within each farm.

In addition, FEC data obtained with the McMaster technique were analysed to characterise the intensity and distribution of GIN infections at both individual and flock level. For each sample, eggs per gram (EPG) were determined for strongylids, *Strongyloides* spp., *Nematodirus* spp., and *Trichuris* spp., and descriptive statistics (minimum, maximum, mean, median, standard deviation, and percentage of positive samples) were calculated across all examined animals. Because FEC data were highly skewed, flock-level infection intensity was summarized using the median strongylid EPG for each flock rather than the mean, and the interquartile range (IQR) was used to describe between-flock variability.

Flocks were classified according to their AR status determined by the MALDT as either resistant or susceptible, and flock-level strongylid FEC median values were compared between BZ- and IVM-resistant and susceptible groups. For graphical presentation, boxplots were generated to display flock-level strongylid FEC medians according to BZ and IVM resistance status. Flock-level strongylid FEC medians were also compared between MDR and non-MDR flocks using the non-parametric Mann-Whitney U test.

3. RESULTS

3.1. Questionnaire survey on worm control practices in Lithuanian sheep farms

3.1.1. General characteristics of surveyed farms

A total of 71 sheep farms participated in the survey (71 / 193), corresponding to a response rate of 36.8% (95% CI: 29.9–44.1%). The largest proportion of respondents was recorded in Utena County (13 / 71; 18.3%), followed by Kaunas and Marijampolė counties (11 / 71; 15.5% each) (Fig. 3.1.1.1). Among the surveyed farms, 60.6% operated under organic management (43 / 71; 95% CI: 48.8–71.5%), whereas 39.4% followed traditional practices (28 / 71; 95% CI: 28.5–51.2%). The mean flock size was 184.5 sheep (range: 10–1600 sheep).

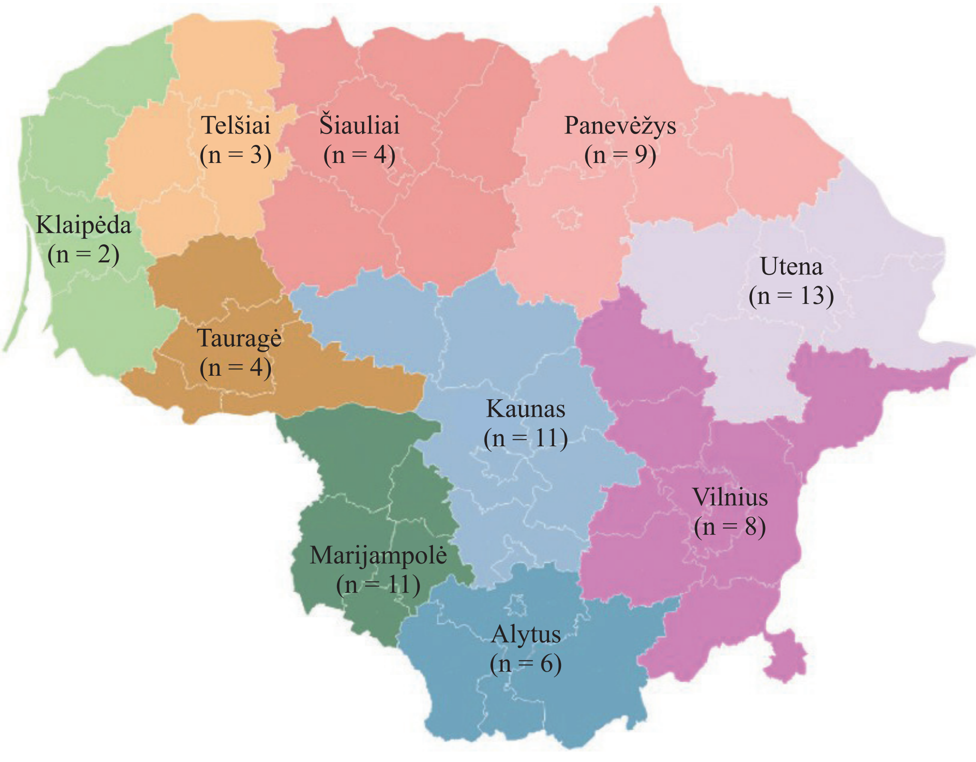


Fig. 3.1.1.1. *Geographic distribution of surveyed sheep farms across the ten counties of Lithuania*

Rotational grazing was reported in 36.6% of farms (26 / 71; 95% CI: 25.6–48.7%), while the remaining 63.4% (45 / 71; 95% CI: 51.3–74.4%) maintained sheep on a single pasture throughout the grazing season. The average pasture area per farm was 34.51 ha (range: 1–200 ha), again highlighting substantial heterogeneity between holdings. When stocking density was considered, the mean number of sheep per ha was 7.4 (median 5.6, interquartile range 3.6–9.8), with values ranging from 1.3 to 31.7 sheep per ha. Based on these values, farms were classified into three stocking density categories: low (< 3 sheep/ha), moderate (3–7 sheep/ha), and high (> 7 sheep/ha). Most farms fell into the moderate category (28 / 71; 39.4%; 95% CI: 28.1–51.9%), whereas 21.1% (15 / 71; 95% CI: 12.5–32.4%) were classified as low-density and an equal proportion (28 / 71; 39.4%; 95% CI: 28.1–51.9%) as high-density holdings.

The Lithuanian black head was the predominant breed, present on 46.5% of farms (33 / 71; 95% CI: 34.9–58.3%), followed by mixed breeds (18.3%; 13 / 71), Merinolandschaf (5.6%; 4 / 71) and Suffolk (5.6%; 4 / 71). Grazing typically commenced in May (34 / 71; 47.9%), less frequently in April (30 / 71; 42.3%) or March (7 / 71; 9.9%), and concluded predominantly in November (54 / 71; 76.1%), with fewer farms extending the period into December (16 / 71; 22.5%) or ending earlier in October (1 / 71; 1.4%). Most farms (87.3%; 62 / 71; 95% CI: 77.9–93.9%) did not keep other livestock species. Of those that did, 8.5% also kept goats (6 / 71), 2.8% cattle (2 / 71), and 1.4% horses (1 / 71), indicating that multi-species grazing systems were relatively uncommon among the surveyed farms. Water supply practices varied, with 67.6% of farms (48 / 71; 95% CI: 55.3–78.2%) relying primarily on well water, while the remainder utilized natural moist areas in pastures supplemented by other on-farm sources

3.1.2. GIN treatment strategies

Nearly half of the farmers relied primarily on their own prior experience when deciding whether GIN was present (40.9%, n = 27), and diarrhoea served as the second most frequently reported indicator, mentioned by roughly one quarter of respondents (Table 3.1.2.1). Objective diagnostic methods, such as faecal examination, were used less often (19.7%, n = 13), and veterinary recommendations played only a minor role, being cited by fewer than 10% (n = 4) of respondents. Only a small number of farmers (n = 5) did not specify any clear decision criterion.

Table 3.1.2.1. *Criteria influencing farmers' decisions regarding assessment of gastrointestinal nematode infection (n = 66)*

Criterion	n	%	95% CI
Prior experience	27	40.9	29.9–53.0
Occurrence of diarrhoea	15	22.7	14.3–34.2
Faecal examination	13	19.7	11.9–30.8
Veterinary recommendations	4	6.1	2.4–14.6

Most farmers administered anthelmintics before turning out to pasture (67.2%, n = 43), whereas treatments before housing or during the housing period were used by only about one-sixth of respondents (15.6%, n = 10). Spring treatments after turnout and dosing before mating or lambing were even less common (7.8%, n = 5 and 4.7%, n = 3, respectively), and only a few farmers reported treating animals when moving them to a new pasture or at multiple time points within the production cycle (together < 5%, n = 3). Additionally, 8.5% of farms (n = 6) reported not administering any anthelmintic treatments (Table 3.1.2.2).

Table 3.1.2.2. *Analysis of anthelmintic treatment timing in sheep farms*

Timing of anthelmintic treatment	n / total	%	95% CI
Before turning out to pasture	43 / 64	67.2	54.9–78.0
Before housing or during the housing period	10 / 64	15.6	7.8–26.9
In the spring after the turnout	5 / 64	7.8	2.6–17.3
Before mating or lambing	3 / 64	4.7	1.0–13.1
When moving animals to a new pasture	1 / 64	1.6	0.0–8.4
At multiple time points within the production cycle	2 / 64	3.1	0.4–10.8
No anthelmintic treatments administered	6 / 71	8.5	3.2–17.5

Overall, 90.2% of sheep farms (n = 64) reported using anthelmintics to control GIN. On average, anthelmintic treatment was administered approximately 1.5 times per year to lambs (< 1 year of age) and older sheep, including yearlings (1–2 years of age) and adult ewes (> 2 years of age), with mean treatment frequencies of 1.54 and 1.49 treatments per year, respectively. Nearly half of the farms treated yearlings / ewes twice per year (49.3%, n = 35), a similar pattern being observed in lambs (43.7%, n = 31), while a substantial proportion of farms received only a single annual treatment (38.0%, n = 27 for both groups). More intensive regimens involving three annual treatments were relatively uncommon (4.2% of farms for yearlings /

ewes and 9.9% for lambs), and 8.5% of farms (n = 6) reported not treating either group at all (Table 3.1.2.3).

Table 3.1.2.3. *Anthelmintic treatment frequency for lambs and yearlings / ewes in surveyed sheep farms (n = 71)*

Group	Number of treatments per year	n	%	95% CI
Yearlings / ewes	1	27	38.0	26.8–50.3
	2	35	49.3	37.2–61.4
	3	3	4.2	0.9–11.9
	Never	6	8.5	3.2–17.5
Lambs	1	27	38.0	26.8–50.3
	2	31	43.7	31.9–56.0
	3	7	9.9	4.1–19.3
	Never	6	8.5	3.2–17.5

When comparing production systems, traditional farms used anthelmintics more frequently than organic farms: yearlings and ewes on traditional farms received on average 1.71 drenches per year, which was approximately 1.27 times higher than in organic farms (1.35 treatments per year, $p < 0.05$), and a similar pattern was observed in lambs, with 1.71 versus 1.44 annual treatments, representing a 1.19-fold increase ($p < 0.05$).

Most farmers estimated anthelmintic doses using subjective methods, most commonly by visual appraisal of animal weight (45.1%, n = 32), while a further 35.2% (n = 25), weighed only a single medium-sized animal and extrapolated this dose to the whole flock. Truly weight-based dosing practices were uncommon: only a small minority of farmers weighed the heaviest animal or each sheep individually (together < 10%, n = 6), and around one in ten farms (11.3%, n = 8) reported not using anthelmintic treatments at all (Table 3.1.2.4).

Table 3.1.2.4. *Anthelmintic dose estimation practices reported by sheep farmers (n = 71)*

Method of dose estimation	n	%	95% CI
Visual appraisal of weight	32	45.1	33.6–56.9
Weighing a medium-sized sheep	25	35.2	24.6–47.2
Weighing the largest sheep	4	5.6	1.5–13.6
Weigh each animal	2	2.8	0.3–9.7
No treatment	8	11.3	5.0–21.0

ML, either used alone or in combination with other anthelmintic classes, was the predominant drug group, being reported by nearly three-quarters of farms (74.6%, n = 53). In contrast, BZ was used on just over one-third of farms (35.2%, n = 25), and LEV was administered much less frequently, by only 12.7% of farms (n = 9), either as a single class or within multi-class regimens. Rotation between BZ and ML was specifically reported by 16.9% of farms (12 / 71), indicating that a minority of farmers actively alternated between drug classes (Table 3.1.2.5).

Table 3.1.2.5. *Anthelmintic classes used rotation patterns in surveyed sheep farms (n = 71)*

Anthelmintic use pattern	n	%	95% CI
ML	36	50.7	39.4–62.0
LEV	4	5.6	1.4–11.3
BZ	8	11.3	4.2–19.7
BZ + ML	12	16.9	8.5–25.4
BZ + ML + LEV	5	7.0	1.4–14.1
No treatment	6	8.5	2.8–15.5

Additionally, 12.7% of farmers (9 / 71) reported importing sheep from foreign countries. For newly introduced animals, regardless of whether they originated from foreign countries or other Lithuanian farms, quarantine measures were implemented in 45.1% of farms (32 / 71), while 54.9% (39 / 71) did not apply quarantine.

Only a small number of farms reported using alternative parasite control strategies, with 8 out of 71 farms (11.3%) indicating at least one non-chemical measure. The most frequently reported alternatives were targeted treatment of selected animals, such as only young or clinically affected sheep (4 / 71; 5.6%), and the use of plants with presumed antiparasitic properties (2 / 71; 2.8%), whereas breeding for increased parasite resistance and culling heavily affected animals were each mentioned by a single farm (1 / 71; 1.4% for both practices).

3.2. Prevalence of GIN

A total of 400 faecal samples collected from 38 sheep flocks were examined using the McMaster technique. Strongylid egg counts showed by far the greatest variability, ranging from undetectable levels to very high burdens (> 8000 EPG), with a median of 60 EPG, indicating that most animals had low to moderate strongylid infections (Table 3.2.1). In contrast, *Nematodirus* spp. and *Trichuris* spp. egg counts were generally very low, with median values of 0 EPG and only occasional samples showing higher counts, and *Strongyloides* spp. reached high EPG values only in a minority of animals. At the population level, strongylid eggs were detected in roughly three-quarters of all examined samples, whereas *Strongyloides* spp., *Nematodirus* spp., and *Trichuris* spp. eggs were present only in a minority of samples, confirming that strongylids were the predominant GIN in the studied flocks (Table 3.2.1).

Table 3.2.1. *Prevalence and EPG counts of gastrointestinal nematodes in the examined samples (n = 400 samples from 38 sheep flocks)*

	Min	Max	Mean	Median	SD	% positive
Strongyloides spp.	0	760	18.77	0.0	58.29	30.0
Nematodirus spp.	0	220	3.45	0.0	18.97	5.5
Strongylids	0	8120	268.40	60.0	839.57	75.2
Trichuris spp.	0	160	1.00	0.0	8.67	3.2

When examined at flock level (Accessory No 2), median strongylid EPG values varied widely between flocks, from 0 to 710 EPG (median 50.0; interquartile range 32.5–117.5; mean of flock medians 110.5 EPG, SD 151.1). A small number of flocks showed very high infection intensities, with median values of 710 EPG (ID 1), 460 EPG (ID 2), and 440 EPG (ID 3), and corresponding mean values up to 1970 EPG in the most heavily infected flock, with maximum individual counts reaching 5400–8120 EPG. In contrast, several flocks had median strongylid EPG equal to 0 (ID 34, 35, 36, 37, 38), with low mean values (13–36 EPG in most of these flocks) and maximum counts not exceeding 200 EPG in four of them.

For the minor nematode groups, flock-level median EPG values were also predominantly 0, even though a proportion of animals in some flocks were positive. Across all flocks, the percentage of animals shedding strongylids eggs ranged from 10% to 100%, whereas the proportion of *Strongyloides*-positive animals usually remained below 40%, and the proportions of *Nematodirus* and *Trichuris*-positive animals were generally even lower (Accessory No 2). In most flocks, the median flock-level EPG for *Nematodirus*, *Strongyloides*, and Trichuris was 0, but the percentage data show that up to one-third of animals in some flocks shed *Strongyloides* eggs and up to 10–20% shed *Nematodirus* or *Trichuris* eggs.

3.3. Prevalence of anthelmintic resistance

MALDT revealed resistance to at least one broad-spectrum anthelmintic class on more than half of the examined farms (57.9%; 22 / 38; 95% CI: 40.8–73.3). Development of L3 at the IVM threshold concentration indicated IVM resistance on 47.4% of farms (18 / 38; 95% CI: 31.0–64.2), while resistance to BZ was detected on 39.5% of farms (15 / 38; 95% CI: 24.0–56.6) (Fig. 3.3.1). Among IVM-resistant farms, moderate resistance was detected on 12 farms (12/18; 66.7%), whereas mild and severe resistance were observed on three farms each (3 / 18; 16.7% in both categories). Among BZ-resistant farms, eight (8 / 15; 53.3%) exhibited moderate resistance, four (4 / 15; 26.7%) showed severe resistance and three (3 / 15; 20.0%) had mild resistance (Fig. 3.3.2). No resistance to LEV was detected in any of the flocks investigated. Notably, BZ resistance occurred on several farms without recent BZ use (8 / 15 BZ-resistant farms), and IVM resistance was observed on one farm where IVM had not been reported.

Fig. 3.3.1. *Prevalence of anthelmintic resistance to BZ and IVM across studied sheep farms*

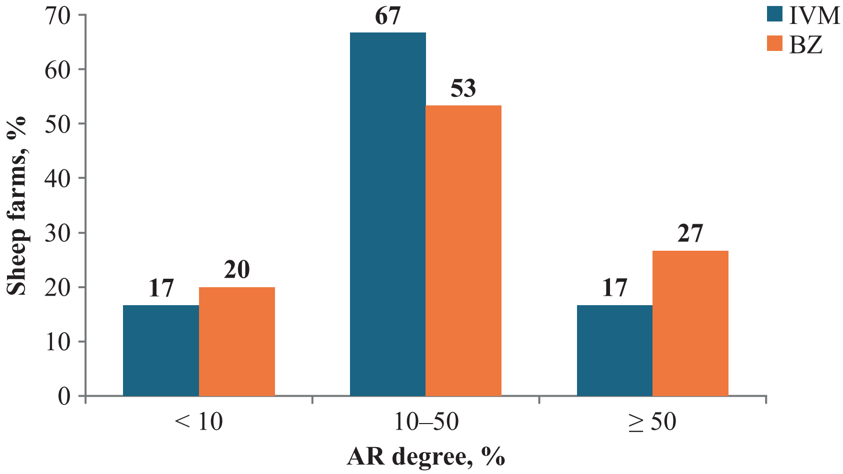


Fig. 3.3.2. *Distribution of sheep farms by degree of AR to IVM and BZ as determined by the MALDT*

3.4. Patterns of single and multiple drug resistance

Of the 38 farms examined, 16 (42.1%) showed no evidence of resistance to any of the tested anthelmintics. Resistance to at least one drug class was detected on the remaining 22 farms (57.9%), with single-class resistance and concurrent resistance to both BZ and IVM (MDR) occurring with equal frequency (each on 11 farms, 28.9%). Among farms with single-class resistance, most were resistant to IVM alone (6 / 11), followed by BZ alone (4 / 11). Overall, MDR accounted for half of all resistant flocks (11 / 22), whereas just over two-fifths of farms remained fully susceptible to all three tested anthelmintic classes (Fig. 3.4.1).

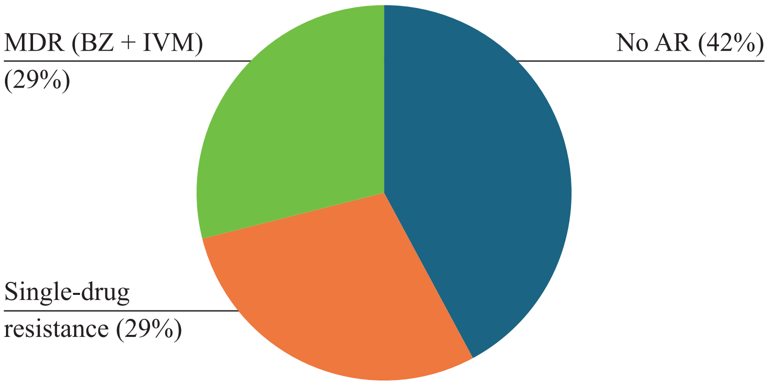


Fig. 3.4.1. *Proportion of sheep farms with no AR, single-drug resistance and MDR based on MALDT results (n = 38 farms)*

3.5. Association between farm factors and anthelmintic resistance

BZ resistance was significantly associated with breed type (p = 0.001), with most BZ-resistant farms maintaining non-local breeds, whereas Lithuanian local and mixed-breed flocks were only rarely affected (Fig. 3.5.1). In contrast, the apparent association between breed type and IVM resistance did not reach statistical significance (p = 0.06) and was therefore not considered supported. Mixed-breed flocks showed no statistically significant association with resistance to either BZ or IVM (p > 0.05).

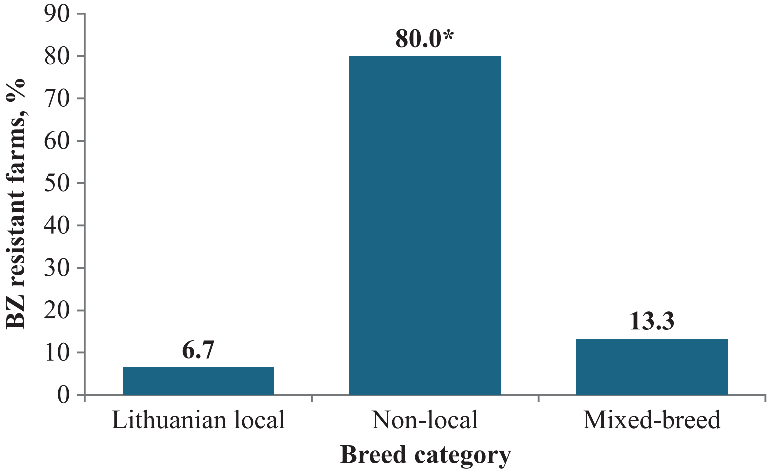


Fig. 3.5.1. *Association between breed type and BZ-resistance on sheep farms (*p = 0.001)*

Furthermore, the use of BZ products was significantly associated with the presence of BZ resistance: most farms using BZ were classified as BZ-resistant (81.8%), compared with only about one-fifth of farms that did not use BZ (22.2%). In contrast, IVM use was not significantly associated with IVM resistance, as similar resistance prevalences were observed in farms that did and did not report IVM use, most likely reflecting the small number of non-using farms. The annual deworming frequency also showed no significant relationship with resistance intensity for any of the tested anthelmintics, with most farms implementing two treatments per year and fewer farms treating once, three times, or not at all. Among farms with valid MALDT data, MDR was detected in 28.9% of flocks and was significantly more common in farms keeping non-local sheep's than in farms with only local breeds (p = 0.007) (Table 3.5.1).

Table 3.5.1. *Analysis of factors associated with anthelmintic resistance in sheep farms (n = 38)*

Factor	Category	Resistant / total farms, n (%)	Statistical association
BZ use *vs* BZ resistance	Uses BZ anthelmintics	9 / 11 (81.8%) BZ-resistant	p = 0.001
	Does not use BZ	6 / 27 (22.2%) BZ-resistant	
IVM use *vs* IVM resistance	Uses IVM	17 / 32 (53.1%) IVM-resistant	p > 0.05*
	Does not use IVM	1 / 5 (20.0%) IVM-resistant	
Annual deworming frequency	0 treatments / year	4 / 38 farms (10.5%)	IVM: p = 0.103 BZ: p = 0.234
	1 treatment / year	8 / 38 farms (21.1%)	
	2 treatments / year	23 / 38 farms (60.5%)	
	3 treatments / year	3 / 38 farms (7.9%)	
MDR *vs* imported sheep	Local sheeps	2 / 20 (10.0%)	
	Non-local	9 / 18 (50.0%)	p = 0.007

*The difference did not reach statistical significance (p > 0.05), likely due to the small number of farms not using IVM (n = 5).

3.6. Association between treatment decision criteria, dosing practices and anthelmintic resistance

The criteria used by farmers to determine the need for anthelmintic treatment were not significantly associated with the occurrence of BZ resistance (p = 0.695), IVM resistance (p = 0.983) or MDR (p = 0.829). Similarly, no statistically significant association was observed between dosing method and the occurrence of BZ resistance (p = 0.149), IVM resistance (p = 0.218) or MDR (p = 0.078) (Table 3.6.1).

Table 3.6.1. *Association between treatment decision criteria, dosing method and anthelmintic resistance in 38 sheep farms*

Variable	Category	BZ-resistant farms, n (%)	IVM-resistant farms, n (%)	MDR farms, n (%)
Treatment decision criterion	Laboratory-based (faecal testing / slaughterhouse data)	2 / 6 (33.3)	3 / 6 (50.0)	1 / 6 (16.7)
	Clinical signs	6 / 12 (50.0)	6 / 12 (50.0)	4 / 12 (33.3)
	Previous experience / recommendations	6 / 15 (40.0)	7 / 15 (46.7)	5 / 15 (33.3)
	No treatment	1 / 5 (20.0)	2 / 5 (40.0)	1 / 5 (20.0)
Dosing method	Accurate (weighing the largest sheep, each animal)	2 / 2 (100.0)	2 / 2 (100.0)	2 / 2 (100.0)
	Non-accurate dosing (visual appraisal, weighing the rages sheep)	13 / 36 (36.1)	16 / 36 (44.4)	9 / 36 (25.0)

3.7. Identification of resistant nematode genera

Differentiation of L3 larvae at the discriminating concentration revealed the nematode genera associated with AR in the investigated flocks. Among BZ-resistant flocks, *Trichostrongylus* spp. was identified on 8 farms (53.3%; 95% CI 26.6–78.7%), *Teladorsagia* spp. on 6 farms (40.0%; 95% CI 16.3–67.7%), and *H. contortus* on 1 farm (6.7%; 95% CI 0.2–31.9%), indicating that BZ resistance was mainly linked to *Trichostrongylus* and *Teladorsagia* infections. In IVM-resistant farms, *Trichostrongylus* spp. was detected on 10 farms (55.6%; 95% CI 30.8–78.5%), *Teladorsagia* spp. on 6 farms (33.3%; 95% CI 13.3–59.0%), and *H. contortus* on 2 farms (11.1%; 95% CI 1.4–34.7%), and no other nematode genera were observed at the discriminating concentration (Fig. 3.7.1). MDR was confirmed on one farm where *H. contortus* was detected, whereas in the remaining MDR cases *Trichostrongylus* spp. (5 / 11 farms; 45.5%; 95% CI 16.7–76.6%) and *Teladorsagia spp*. (5 / 11 farms; 45.5%; 95% CI 16.7–76.6%) were the predominant genera.

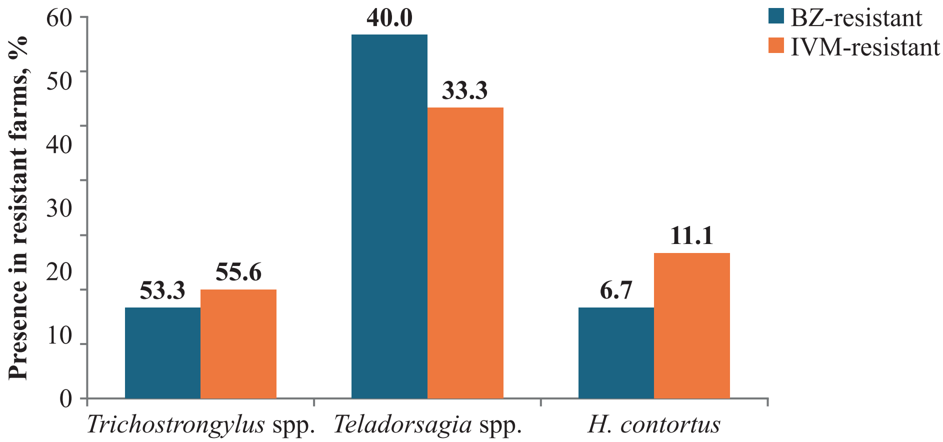


Fig. 3.7.1. *Nematode genera identified in resistant farms*

3.8. Association between faecal egg counts and anthelmintic resistance

To explore whether flock-level infection intensity was associated with AR, McMaster results were compared between flocks classified as resistant or susceptible based on *in vitro* results. Flocks with BZ resistance (n = 15) had higher median strongylid egg counts than BZ-susceptible flocks (n = 23): the median flock-level strongylid EPG was 90 (range 0–710) in BZ-resistant flocks compared with 40 EPG (range 0–320 EPG) in flocks without detected BZ resistance (Fig. 3.8.1). Similarly, flocks with IVM resistance (IVM-R; n = 18) showed higher median strongylid egg counts than IVM-susceptible flocks (IVM; n = 20), with median values of 75 EPG (range 0–710 EPG) and 40 EPG (range 0–440), respectively (Fig. 3.8.2).

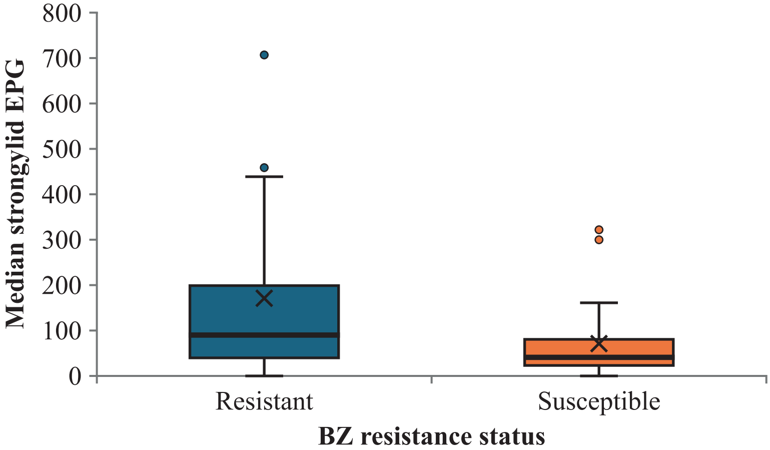


Fig. 3.8.1. *Flock-level median strongylid EPG by BZ resistance status in 38 sheep flocks in Lithuania*

The horizontal line inside the box indicates the median, the "×" symbol represents the mean, and the dots indicate outliers.

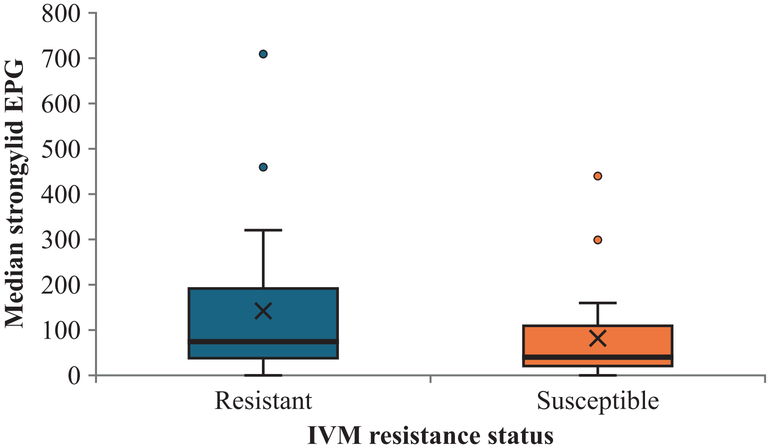


Fig. 3.8.2. *Flock-level median strongylid EPG by IVM resistance status in 38 sheep flocks in Lithuania*

The horizontal line inside the box indicates the median, the "×" symbol represents the mean, and the dots indicate outliers.

Flocks with MDR ($n = 11$) had higher flock-level median strongylid FEC than non-MDR flocks (median 90 EPG, IQR 50–195 *vs* 40 EPG, IQR 25–95; Mann-Whitney $U = 201$, $p = 0.093$), although this difference did not reach conventional statistical significance (Fig. 3.8.3).

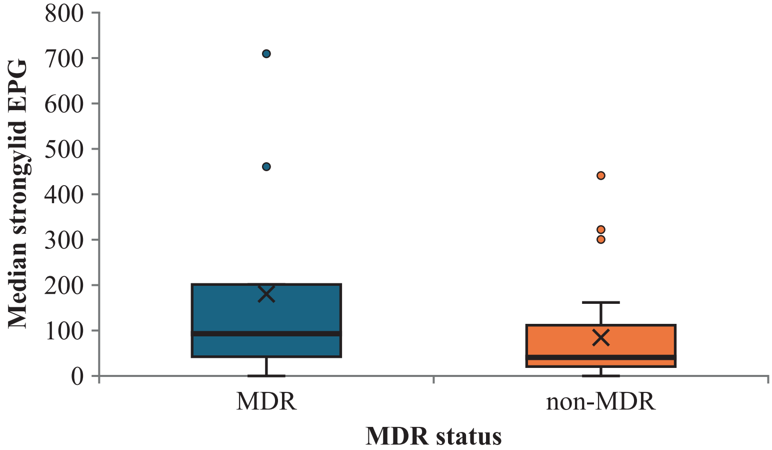


Fig. 3.8.3. *Flock-level median strongylid EPG according to multidrug resistance status in 38 Lithuanian sheep flocks*

The horizontal line inside the box indicates the median, the "×" symbol represents the mean, and the dots indicate outliers.

4. DISCUSSION

This study provides the first nationwide, population-based assessment of anthelmintic resistance and parasite control practices in Lithuanian sheep farms, integrating questionnaire data, FEC, and micro-agar larval development tests. Our findings demonstrate that resistance to IVM and BZ is already established on a substantial proportion of farms, while LEV remains fully effective (0 / 38 farms with detected resistance; 95% CI 0.0–9.3%). This confirms that the Lithuanian situation now resembles broader European trends but with some important regional specificities. Taken together, these results suggest that current control practices are unlikely to be sustainable in the long term and provide an evidence base for developing more integrated, risk-based parasite management strategies in Lithuanian sheep farming.

4.1. Parasite control strategies and anthelmintic use on Lithuanian sheep farms

The results of the present study provide valuable information on parasite control strategies currently implemented on sheep farms in Lithuania. Although the questionnaire response rate among members of the Lithuanian Sheep Breeders Association was 36.8%, the collected responses still constitute a meaningful dataset that provides an overview of existing parasite management practices and highlights potential areas for improvement. Nevertheless, the moderate participation rate may reflect several common barriers to farmer engagement in surveys, including limited time availability, lack of interest in research activities, or concerns regarding the confidentiality of shared information. Furthermore, some sheep farmers may not consider GIN infections a major problem on their farms, which could reduce their motivation to participate in studies on parasite control.

Regarding grazing management, only 36.6% of surveyed farms reported practicing rotational grazing, whereas most farms (63.4%) kept sheep on a single pasture with access to shelter throughout the grazing season. A relatively small proportion of farmers (12.7%) reported implementing mixed or alternate grazing with cattle or horses. This approach is widely recognized as an effective strategy for lowering parasite contamination on pastures and reducing dependence on anthelmintic treatments in parasite control programs [110]. Only 11.3% of farms reported using at least one non-chemical parasite control strategy, such as mixed or alternate grazing, targeted selective treatment, or genetic selection for increased parasite resistance. This low uptake of alternative measures highlights a key opportunity to improve the long-term sustainability of parasite control programs.

The results also demonstrate that parasitological diagnostic methods are used relatively infrequently by Lithuanian sheep farmers, particularly in traditional farming systems. Treatment decisions in these farms, therefore, appear to be less evidence-based. Traditional farms also reported higher drenching frequencies, suggesting a more intensive and possibly prophylactic treatment approach, which may contribute to the selection and development of anthelmintic resistance. Only 18.3% of respondents reported using faecal parasitological examinations to guide treatment decisions, while most farmers relied on previous experience (38.0%) or observable clinical signs, such as diarrhoea (22.5%). Similar tendencies have been reported in several other European countries [26, 111, 112]. For example, in Belgium, only a small proportion of sheep farmers (9%) routinely applied FEC monitoring to assess parasite infections [49]. This reliance on subjective indicators rather than on objective diagnostic testing highlights an important area for improving parasite control practices in Lithuania. Wider implementation of FEC monitoring could enable more targeted anthelmintic use, potentially reduce treatment frequency, and slow the development of AR. Additional analysis showed that, in this subset of 38 farms, the main treatment decision criterion (laboratory testing, clinical signs or previous experience) was not significantly associated with the occurrence of BZ resistance, IVM resistance or MDR (all $p > 0.05$). This suggests that resistance is already widespread across different types of farms, regardless of whether treatment decisions are based on objective diagnostic tests or more subjective criteria.

Evidence from other countries suggests that farmers may underestimate the importance of diagnostic testing. For instance, a study conducted in Norway found that approximately 90% of farmers did not suspect reduced treatment efficacy [26], indicating that established parasite control routines are rarely questioned. If treatments appear to provide acceptable results, farmers may consider additional diagnostic testing unnecessary and perceive it as an extra cost. In practice, farmers must balance numerous management priorities, including animal welfare, farm management, and regulatory compliance. Under such circumstances, parasitological testing may not be regarded as a priority, especially when anthelmintic treatment seems to be effective. In addition, insufficient encouragement or recommendations from veterinarians may also contribute to the low use of diagnostic testing.

Previous research by Kupčinskas et al. [17] reported that 92.2% Lithuanian sheep farmers determined the appropriate anthelmintic dose by visually estimating animal body weight. The present findings suggest that this situation has improved to some extent, as visual weight estimation is currently reported by a smaller proportion of farmers (45.1%). Similar dosing practices have also been documented in Norway, Sweden, Slovakia, and France, where visual estimation of animal weight remains the most used method for determining treatment doses [29, 105, 113, 114]. However, inaccurate estimation of live weight and improper calibration of dosing equipment may result in underdosing. To minimize this risk, body weight should ideally be measured individually with a scale. Alternatively, weighing the heaviest animal in the group and slightly overestimating the dose for all animals can help ensure that sufficient anthelmintic doses are administered [105]. In the present survey, when only individual weighing or weighing of the heaviest animal was considered acceptable for accurate dose determination, only 8.5% of sheep flocks applied correct dosing practices. Burgess et al. [115] suggested that underdosing with anthelmintics from the same drug class may pose a greater risk for the development of resistance than the frequency of drenching itself, which in this study appeared relatively low.

Compared with earlier findings in Lithuania [17], the overall use of anthelmintics has increased substantially, rising from 71.8% to 90.2% of farms. Despite this increase, the mean annual drenching frequencies for lambs and ewes have only slightly increased, from 1.24 to 1.54 treatments for lambs and from 1.29 to 1.49 treatments for ewes. These treatment frequencies remain somewhat lower than those reported in many other European countries. For example, mean annual drenching frequencies of 2.5 for lambs and 1.9 for ewes have been reported in Norway [105], while in Denmark the respective values were 1.9 for lambs and 2.3 for ewes [116]. In the Netherlands, lambs were treated on average 2.05 times per year, and ewes 1.53 times per year [112]. Even higher treatment frequencies have been observed in Belgium, where lambs and ewes were treated on average 3.2 and 2.6 times per year, respectively [49]. In the United Kingdom, lambs typically receive between 2.2 and 4.4 treatments annually [89, 111], whereas in France, an average treatment frequency of 5.2 times per year has been reported [117]. In contrast, somewhat similar treatment frequencies to those observed in the present study have been documented in Slovakia (1.70–1.76 treatments per year) [114]. Sweden represents an example of comparatively low anthelmintic use, where only 45% of farms reported using these treatments, and most ewes were treated only once per year [29]. Frequent anthelmintic treatments are widely recognized as an important risk factor contributing to the development of AR [118]. In addition, previous studies have shown that farmers who treat their ewes more frequently also tend to administer treatments more frequently to lambs [112]. Even though, on average, lambs and ewes in this study received only about 1.5 treatments per year, which is lower than the 2–5 annual drenches commonly reported from several other European countries, the overall anthelmintic treatment frequency in Lithuanian sheep flocks can still be regarded as relatively low in a broader European context.

Another notable observation from this study is the relatively high proportion of organic sheep farms (60.6%) compared with traditional production systems (39.4%), suggesting that sustainable and environmentally oriented farming approaches are widely adopted in Lithuania. Organic farms were more likely to use parasitological diagnostics, with 23.3% of farmers reporting faecal analysis, compared with only 10.7% of traditional farms. These differences indicate variation in parasite-control strategies across production systems. The finding that traditional farms tend to administer anthelmintics more frequently than organic farms likely reflects fundamental differences in disease management approaches. Organic farming systems generally emphasize the development of natural resistance and the use of preventive, management-based strategies (e.g. grazing management, appropriate breeding and nutrition) rather than routine chemotherapy. This is reflected in lower frequencies of antiparasitic treatments and a stronger focus on non-chemical or targeted control options reported in organic small ruminant systems. In contrast, conventional production systems have been shown to rely more heavily on systematic, often calendar-based anthelmintic treatments to control parasitic infections [119, 120].

The present study also demonstrated that, when FEC were measured systematically using the modified McMaster method, strongylids infections were highly prevalent and often occurred at potentially clinically relevant intensities, even on farms where parasitological testing was not routinely used for decision-making. Across all samples from 38 flocks, strongylids eggs were detected in 75.2% of faecal samples, with flock-level median strongylid EPG values ranging from 0 to 710 EPG, and some farms showed mean values close to 2000 EPG. Similar or even higher prevalences and burdens of strongylid infections have been reported in sheep flocks in other European countries, where most animals carry low-to-moderate infections, but a subset of flocks or animal groups exhibit substantially elevated EPG levels [121]. The large between-flock variation in strongylid EPG observed in the McMaster data, with some farms showing median counts of 0 EPG while others exceeded 400–700 EPG, illustrates how flock-specific monitoring could help identify high-risk farms and tailor parasite control strategies accordingly, particularly in systems that currently rely little on diagnostics. These findings suggest that relying solely on clinical signs or previous experience may underestimate the true burden of GIN infections and are consistent with previous work emphasizing the importance of routine FEC monitoring as a core component of parasite-control programs in small-ruminant flocks [122, 123].

Among the available anthelmintic drug classes, ML were the most used on Lithuanian sheep farms, with 50.7% of respondents reporting their use. However, this represents a slight decrease compared with earlier findings, where ML use was reported by 56.9% of farmers [17]. The use of BZ has also declined, from 15.7% [17] to 11.3% in the present study. In contrast, the use of LEV has increased slightly, from 3.9% [17] to 5.6%, although it remains relatively infrequent. These trends may indicate continued reliance on a limited number of anthelmintic classes, which could accelerate the development of resistance. The selection of anthelmintics in Lithuania appears to resemble patterns observed in Northern European countries but differs from some other European regions, where ivermectin is increasingly used alongside compounds such as closantel, doramectin, MOX, and combination treatments [17, 29, 124]. Anthelmintic rotation was reported by only 23.9% of farms, and IVM, BZ, and LEV remain the only single active substances registered for the control of sheep GIN infections in Lithuania. At the same time, strict animal welfare regulations are in place, and anthelmintic drugs can only be obtained with a veterinary prescription. Taken together, these findings indicate that Lithuanian sheep farms rely heavily on a small number of anthelmintic classes, particularly IVM-based ML, and that this usage pattern is consistent with the resistance profiles later demonstrated by MALDT, in which reduced susceptibility to BZ and ML was frequently observed.

Quarantine measures are widely recognized as an important component of sustainable parasite control strategies and play a key role in preventing the introduction and spread of anthelmintic-resistant parasite populations [26, 27]. The results of this survey indicate that Lithuanian farmers commonly administer anthelmintic treatments to newly introduced animals, which is consistent with practices reported in Norway [26], Sweden [29], and the Netherlands [30]. The movement and trade of sheep both within Lithuania and between countries may therefore represent an important pathway for the dissemination of resistant parasite populations. Given the documented role of animal movements in introducing resistant nematodes, the reported quarantine practices should be interpreted considering the AR patterns identified in the present study, which suggest that the importation of resistant parasites is a realistic concern for Lithuanian sheep farms

4.2. Anthelmintic resistance patterns in Lithuanian sheep farms

A 41-year analysis of AR trends in sheep nematode populations across Europe demonstrated a progressive increase in resistance to BZ, while resistance to ML has risen more rapidly in recent years [5]. The present research represents the first nationwide study in Lithuania in which sheep farms were recruited sequentially from the complete national registry, without pre-selection based on suspected resistance and without restrictions related to previous anthelmintic treatment practices. Such an approach is important because methodological design can strongly influence estimated prevalence rates of AR. As highlighted by Vineer et al. [5] studies based on non-random sampling may overestimate resistance levels when farms are included because researchers suspect AR, or when farmers volunteer to participate due to concerns about treatment failure. In many cases, AR studies have been conducted on a limited number of farms near universities or research centres, with a median sample size of only 5 farms in investigations of GIN resistance [15].

In addition to providing prevalence estimates of AR, the present study also allowed an assessment of how resistance status related to flock-level infection intensity. Using McMaster FEC data, flock-level median strongylid EPG values were compared between farms classified as resistant or susceptible to BZ and IVM. Flocks with documented BZ or IVM resistance tended to have higher median strongylid egg counts than susceptible flocks (90 *vs* 40 EPG for BZ; 75 *vs* 40 EPG for IVM), although there was substantial overlap in EPG ranges between groups. These results indicate that higher GIN burdens are more frequently observed in resistant flocks but also confirm that FEC data alone are insufficient to reliably identify AR and must be interpreted alongside specific resistance tests such as FECRT or *in vitro* assays [66, 123, 125].

IVM resistance in Lithuania, using the MALDT method, was assessed during 2013–2014 and identified in 13 of 21 farms (61.9%). In the same research, benzimidazole resistance determined using the EHT was detected on all investigated farms [17]. Another study conducted in 2014 using MALDT reported BZ resistance on 12 of 17 farms (70.6%) and LEV resistance on two of six farms (33.4%) [16]. However, these earlier investigations were geographically limited to central and southern Lithuania and mainly included farms with a known history of anthelmintic treatment [16, 17]. In contrast, the results of the present study suggest a lower occurrence of resistance to BZ and IVM, and no resistance to LEV was identified. This finding is in agreement with reports from northern Europe indicating that levamisole continues to be highly effective against sheep gastrointestinal nematodes [5, 96]. The lower levels of AR observed in the current study may also be explained by the randomized selection of farms. In fact, one of the few randomized surveys conducted in Europe found that resistance to BZ was detected less frequently in randomly selected farms than in farms selected using other sampling approaches – for example, 10.5% versus up to 80% on non-randomly selected farms in Norway [15]. The McMaster results from the present study are consistent with this pattern: despite relatively modest overall treatment frequencies, a considerable proportion of flocks harboured moderate-to-high strongylid burdens. This supports the view that suboptimal parasite control practices, rather than an absence of infection pressure, underline the observed resistance profiles.

An interesting observation in the present study was that BZ resistance was detected on eight farms where farmers reported not using BZ during the previous three years. A similar discrepancy was observed on one farm where ivermectin resistance was detected despite the reported absence of recent IVM use. Comparable findings have been reported from goat farms in Poland, where resistance was detected in three herds to LEV, nine to ML, and three to BZ [20]. According to Mickiewicz et al. [20], such inconsistencies may arise because farmers are sometimes unaware of the specific anthelmintics used on their farms or may not accurately recall previous treatments. Another explanation could be the introduction of animals that had been treated with these drugs before arriving on the farm. The current questionnaire survey also indicated that Lithuanian farmers are not always fully informed about the anthelmintics used in their flocks, as 10.5% of respondents reported either not performing routine deworming or not remembering whether anthelmintics had been used. Considering the relatively small geographical size of Lithuania and the frequent movement of sheep between farms, the introduction of resistant parasite populations from other farms with unknown treatment histories is highly plausible.

Another factor that may contribute to resistance patterns is cross-resistance, a phenomenon in which parasites can tolerate therapeutic doses of chemically unrelated drugs or compounds with different mechanisms of action [53]. Although the mechanisms of resistance to BZ, LEV, and ML differ and cross-resistance between these drug classes is generally considered unlikely [126–128]. Some interactions between resistance mechanisms have been suggested. For example, a study on *H. contortus* demonstrated that exposure to MLs may be associated with an increased frequency of β-tubulin alleles linked to benzimidazole resistance [128]. This finding indicates that the use of ML could potentially facilitate the development of BZ resistance in certain parasite populations.

The occurrence of MDR among GIN remains an important concern for parasite control in Lithuania. Previous research using the FECRT identified MDR to BZ and ML on five of 13 sheep farms (38.5%) [17]. In the present study, MDR involving IVM and BZ was detected on 11 of 38 farms (28.9%) using the same method. Although the proportion of farms affected by MDR appears somewhat lower than previously reported, this difference may be related to the larger sample size included in the current study. Despite this variation, MDR remains a persistent challenge and reflects a pattern documented in many other countries. MDR in GIN has been reported in Scotland, the United Kingdom, Ireland, Denmark, Norway, France, Greece, Italy, and Austria [39, 40, 83, 85, 100, 129, 130]. These studies collectively demonstrate that MDR in nematodes is a widespread and growing global issue. In the present study, MDR flocks (n = 11) also tended to have higher flock-level median strongylid EPG than non-MDR flocks (n = 27), with median values of 90 EPG (IQR 50–195) and 40 EPG (IQR 25–95), respectively, although this difference did not reach conventional statistical significance (Mann-Whitney U = 201, p = 0.093). This pattern suggests that MDR is more frequently associated with moderate to high strongylid burdens, but that infection intensity alone cannot reliably distinguish MDR from non-MDR farms.

The parasite genera most frequently associated with resistance in Lithuanian sheep flocks are *Trichostrongylus*, *Teladorsagia*, and *Haemonchus*. Earlier research conducted in Lithuania identified *Teladorsagia* and *Trichostrongylus* species in all MALDT tests performed [24]. Similar findings have been reported across Europe, where resistant populations commonly involve genera such as *Haemonchus*, *Teladorsagia, Trichostrongylus*, *Cooperia*, and *Nematodirus* [5, 7, 42]. Although this study was not primarily designed to characterise species composition in detail, our results align with previous Baltic reports indicating that *Teladorsagia*, *Trichostrongylus,* and, increasingly*, H. contortus* are among the dominant strongylid genera in sheep. In the context of climate change, with milder winters and longer grazing seasons predicted for northern Europe, the epidemiological importance of *H. contortus* is likely to increase, particularly on farms with intensive grazing and high stocking densities. Under such conditions, existing AR problems may be exacerbated, and the preservation of effective drug classes such as levamisole will become even more critical.

The participation rate in this study was relatively low, with only 38 of the 193 invited farms (19.7%) agreeing to participate. While such response rates are common in voluntary farmer surveys, this limitation should be considered when interpreting the results. Low participation may be due to the demanding nature of sheep farming, which often leaves farmers with limited time to engage in research. In addition, concerns about data confidentiality or uncertainty about how the information will be used may reduce willingness to participate. External factors may also have influenced engagement. During the data collection period, many Lithuanian sheep farmers were actively involved in discussions regarding wolf population management, which was perceived as a more immediate concern than participation in scientific studies.

Taken together, the resistance patterns revealed by MALDT and FECRT, combined with the McMaster FEC data, indicate that Lithuanian sheep farms are already experiencing non-trivial levels of both drug resistance and infection intensity, and that improvements in parasite control practices are urgently needed to prevent further escalation of MDR.

CONCLUSIONS

1. Anthelmintic doses were mostly determined based on visual estimation of animal body weight (45.1%). Quarantine measures for newly purchased animals were often not implemented, with most farmers choosing to administer anthelmintic treatment without prior quarantine (54.9%). In addition, parasitological faecal examinations were conducted in only a small proportion of farms (19.7%).
2. Strongylid infections were present in all examined flocks and were the predominant nematode group in terms of both prevalence and infection intensity. The prevalence of *Strongyloides* spp., *Nematodirus* spp., and *Trichuris* spp. was low.
3. No resistance to LEV was detected in any of the examined flocks. However, resistance to IVM (47.4%) and/or BZ (39.5%) was detected in more than half of the flocks and was most commonly of moderate severity. MDR was detected in 28.9% of flocks. Resistant nematode populations were predominantly composed of *Trichostrongylus* spp., *Teladorsagia* spp., and *H. contortus*.
4. The use of BZ was significantly associated with a higher occurrence of resistance to this drug class, particularly in flocks of non-local sheep breeds ($p = 0.001$). MDR and BZ resistance were detected significantly more frequent in flocks of non-local sheep breeds than in flocks of local breeds ($p = 0.007$ and $p = 0.001$, respectively). The criteria used for treatment decisions and the method of anthelmintic dose calculation were not significantly associated with the occurrence of BZ resistance, IVM resistance, or MDR ($p > 0.05$).

PRACTICAL RECOMMENDATIONS

1. FEC should be monitored at least once or twice during the grazing season, and treatment decisions should be based on the results.
2. The choice of anthelmintic class should be based on the anthelmintic resistance status of the flock and the results of faecal egg count examinations.
3. Doses of anthelmintics should be calculated based on the weight of the heaviest sheep in the group or by individually assessing the body weight of each animal.
4. The use of LEV should be considered in flocks where resistance to BZ or IVM has been confirmed.
5. All newly introduced animals should undergo quarantine, during which faecal examinations should be performed and, if necessary, anthelmintic treatment should be administered before the animals are released onto communal pastures.

SUMMARY IN LITHUANIAN

SANTRUMPOS

AA	–	atsparumas atihelmintikams
BZ	–	benzimidazolas
DA	–	dauginis atsparumas
DMSO	–	dimetilsulfoksidas
IQR	–	tarpkvartilinis intervalas (angl. *interquartile range*)
IVM	–	ivermektinas
IVM-Ag	–	ivermektino agliukonas
KSG	–	kiaušinėlių skaičius grame
L1	–	pirmos stadijos lerva
L2	–	antros stadijos lerva
L3	–	trečios stadijos lerva
LEV	–	levamizolis
LVMAT	–	lervų vystymosi mikroakare testas
ML	–	makrocikliniai laktonai
PI	–	pasikliautinas intervalas
TBZ	–	tiabendazolas
VTN	–	virškinimo trakto nematodai

ĮVADAS

Avių auginimas yra svarbi daugelio Europos šalių gyvulininkystės sektoriaus sudėtinė dalis, prisidedanti prie kaimo ekonomikos, kraštovaizdžio tvarkymo ir agrobiologinės įvairovės [1]. Tačiau avių auginimo pelningumą ir tvarumą smarkiai riboja virškinimo trakto nematodai (VTN), kurie tebėra vieni iš labiausiai paplitusių ir didžiausią poveikį darančių parazitų tarp atrajotojų [2, 3]. Net jei klinikinių požymių nėra, VTN infekcijos mažina kūno svorio prieaugį, pašarų panaudojimo efektyvumą ir skerdenos kokybę bei gali pabloginti reprodukcinius rodiklius, o tai lemia didelius ekonominius nuostolius [4–6].

Svarbiausi avių VTN priklauso *Strongylida* būriui, o tarp jų *Teladorsagia circumcincta* (*T. circumcincta*), *Trichostrongylus* spp. ir *Haemonchus contortus* (*H. contortus*) pripažįstami kaip vyraujantys ir labiausiai patogeninės rūšys Europos vidutinio klimato regionuose [3, 7]. Jų epidemiologiją lemia parazitų biologijos, klimato, ganymo praktikos ir šeimininko imuniteto sąveika. Klimato kaita – dėl kylančių temperatūrų ir pasikeitusio kritulių kiekio – turėtų pailginti ganymo sezoną Šiaurės Europoje ir sudaryti palankias sąlygas labai patogeninėms rūšims, pvz., *H. contortus*. Šie nematodai taip pat dažniausiai siejami su atsparumu antihelmintikams (AA), o avių bandose Europoje nuolat pranešama apie nematodus, atsparius benzimidazolams (BZ), makrocikliniams laktonams (ML) ir levamizolui (LEV), o dauginis atsparumas (DA) nustatomas, remiantis tyrimų duomenimis, mažiausiai dešimtyje šalių, nors tikrasis jo paplitimas tikriausiai yra didesnis nei šiuo metu žinoma [5, 6, 10].

Kadangi atsparumas BZ, ML ir vis dažniau LEV tapo plačiai paplitęs smulkiųjų atrajotojų VTN tarpe, tikslus AA nustatymas ir stebėsena tapo būtini siekiant užtikrinti tvarią kontrolę [1, 5, 10]. *In vitro* tyrimai vis dažniau naudojami AA nustatyti: kiaušinėlų išsiritimo testas daugiausiai taikomas BZ atsparumui nustatyti, o lervų vystymosi testas, įskaitant lervų vystymosi mikroagare testą (LVMAT), padeda nustatyti atsparumą BZ, LEV ir ML, tačiau reikalauja daugiau sąnaudų ir išteklių laboratorije. Šie metodai pasižymi dideliu analitiniu jautrumu ir leidžia aptikti atsparias subpopuliacijas net tada, kai jos sudaro tik nedidelę parazitų populiacijos dalį [11, 12]. Atsižvelgiant į didėjantį AA poveikį, tarptautinėse gairėse dabar akcentuojamos integruotos parazitų valdymo strategijos, kuriose diagnostinis stebėjimas derinamas su tiksliniais atrankiniais gydymo būdais, išlaikymu *refugia*, tiksliu antihelmintikų dozavimu, geresniu ganyklų valdymu ir tinkamomis karantino priemonėmis, kad mažinti atsparumo atihelmintikams vystymąsi ir kartu užtikrinti gyvūnų sveikatą bei produktyvumą [2, 13, 14].

Smulkių atrajotojų bandose atlikti AA tyrimai dažnai pasižymi ribotu imties dydžiu ir tiksline atranka. Dažniausiai tiriami patogiai prieinami arba savanoriškai dalyvauti sutikę ūkiai, neretai esantys netoli mokslinių tyrimų institucijų. Tokia atranka gali iškreipti AA paplitimo vertinimus ir riboti rezultatų pritaikomumą platesnei populiacijai [5]. Tyrimai Norvegijoje parodė, kad atsitiktinai atrinktose bandose atsparumas BZ nustatomas rečiau nei tikslingai pasirinktose, o tai rodo, kad tyrimo shema daro reikšmingą įtaką gautiems rezultatams [15]. Lietuvoje iki šiol atlikti *in vitro* ir *in vivo* tyrimai taip pat buvo ribotos apimties – daugiausia tirti centrinėje ir pietinėje šalies dalyje esantys ūkiai, kuriuose anksčiau buvo intensyviai naudojami antihelmintikai [16, 17].

Nepaisant didėjančios antihelmintinio atsparumo reikšmės avių auginimui, duomenų apie jo paplitimą Lietuvoje vis dar nepakanka. Ribotas ankstesnių tyrimų mastas ir informacijos apie parazitų kontrolės praktikas stoka išryškina poreikį išsamiai įvertinti antihelmintinio atsparumo paplitimą Lietuvos avių bandose bei nustatyti veiksnius, galinčius prisidėti prie jo formavimosi.

Darbo tikslas

In vitro lervų vystymosi mikroagare testu (LVMAT) ištirti virškinimo trakto nematodų (VTN) atsparumo antihelmintikams (AA) paplitimą ir lygį Lietuvos avių bandose bei nustatyti ūkio valdymo ir gydymo veiksnius, susijusius su atsparumo vystymusi.

Tyrimo uždaviniai

1. Remiantis ūkininkų apklausos duomenimis, nustatyti ūkių valdymo ir gydymo praktikas, susijusias su AA atsiradimu Lietuvos avių bandose.
2. Nustatyti VTN paplitimą ir infekcijos intensyvumą tirtose Lietuvos avių bandose.
3. Nustatyti AA paplitimą ir jo lygį pagrindinėms antihelmintinių vaistų klasėms (IVM, BZ, LEV) taikant LVMAT bei identifikuoti labiausiai atsparias pagrindines VTN gentis.
4. Išanalizuoti sąsajas tarp antihelmintinio atsparumo ir avių ūkių charakteristikų bei ūkyje naudojamų antihelmintikų klasių.

Mokslinis naujumas

Buvo įvertintas avių VTN atsparumo BZ, IVM ir LEV paplitimas, taikant sistemingą ūkių atranką – ūkiai atrinkti iš viso Lietuvos avių augintojų asociacijos narių sąrašo, be išankstinės atrankos pagal įtariamą atsparumą. Skirtingai nuo ankstesnių Lietuvoje atliktų AA tyrimų [16, 17], kurie apsiribojo nedideliu skaičiumi tikslingai pasirinktų ūkių, intensyviai naudojančių antihelmintikus, šiame darbe laikytasi griežtesnių epidemiologinės AA stebėsenos rekomendacijų [5]. LVMAT duomenys buvo suderinti su apklausos duomenimis ir taip pirmą kartą sistemingai įvertinta, kaip konkrečios gydymo ir ūkio valdymo praktikos susijusios su stebimais atsparumo modeliais Lietuvos avių bandose.

Praktinė reikšmė

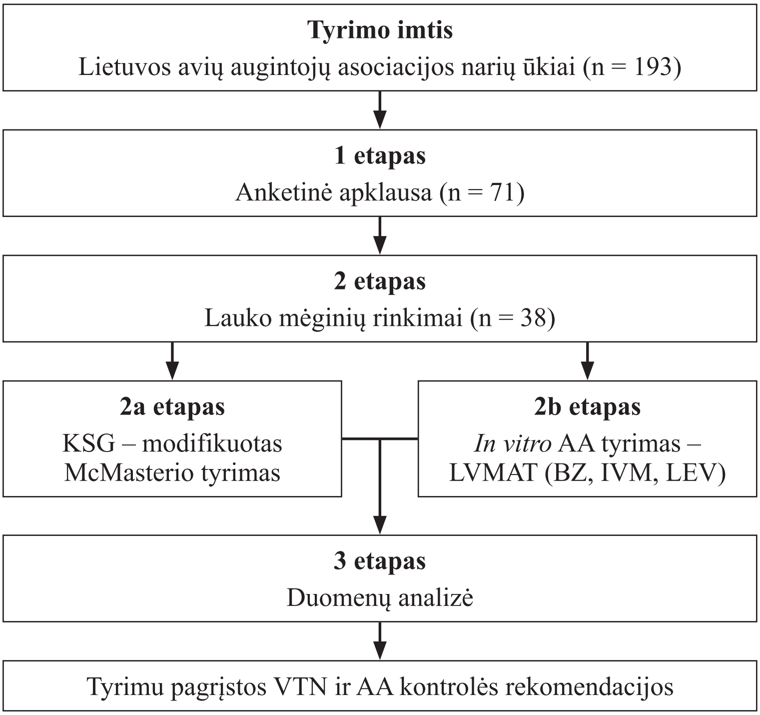
Tyrime nustatyti avių nematodų atsparumo BZ, IVM ir LEV lygiai ir jų sąsajos su konkrečiomis gydymo bei ūkio valdymo praktikomis. Šie rezultatai sudaro pagrindą racionalesniam antihelmintinių preparatų naudojimui, remia integruotų, rizika grindžiamų parazitų kontrolės strategijų, pritaikytų Lietuvos sąlygoms, diegimą ir pateikia praktines rekomendacijas, kaip gerinti bandų sveikatą, išsaugoti vaistų veiksmingumą ir didinti avių auginimo tvarumą.

2. TYRIMO METODAI IR MEDŽIAGA

Tyrimai buvo atlikti Lietuvos sveikatos mokslų universiteto Veterinarijos akademijos Veterinarijos fakulteto Veterinarinės patobiologijos katedroje 2018–2025 m. Tyrimai buvo atliekami laikantis Lietuvos gyvūnų gerovės taisyklių (Nr. B1-866, 2012; Nr. XI2271, 2012) ir buvo patvirtinti Lietuvos veterinarijos ir zootechnikos mokslų komiteto (Protokolas Nr. 07/2010).

Šie tyrimai buvo finansuoti Lietuvos žemės ūkio ministerijos mokslinių tyrimų projekto Nr. MT-18-6. Stažuotės ir dalyvavimas konferencijose remti ES COST projekto Nr. CA16230 – kovos su atrajotojų atsparumu antihelmintikams (COMBAR).

2.1. Tyrimo etapai



2.1.1 pav. *Srautinė tyrimų atlikimo diagrama*

2.2. Apklausa

Norint surinkti informaciją apie taikomą avių auginimo praktiką Lietuvos avių augintojų asociacijos narių ūkiuose, buvo atlikta skerspjūvio (*cross-sectional*) anketinė apklausa. Apklausa vykdyta 2022–2023 m. Į apklausą buvo įtraukti visi 193 asociacijos nariai. Duomenys buvo renkami respondentus apklausiant tiesiogiai, telefonu. Iš viso anketą užpildė 71 respondentas (36,8 proc.), o likę asociacijos nariai į pakartotinius skambučius arba neatsiliepė, arba dalyvauti apklausoje atsisakė. Prieš interviu buvo gautas žodinis sutikimas, o vėliau visiems dalyviamsbuvo išsiųsta rašytinė sutikimo forma.

Anketa buvo adaptuota ir modifikuota remiantis COST veiksmų programos COMBAR parengtu klausimynu. Pakeitimai buvo atlikti atsižvelgiant į šio tyrimo tikslus ir Lietuvos avių ūkių specifiką. Galutinę anketą sudarė 16 klausimų. Pagrindinės klausimų kryptys: (1) bendrosios ūkio charakteristikos (4 klausimai); (2) ganymo valdymas (3 klausimai), susijęs su ganymo trukme ir sistema; ir (3) VTN kontrolės strategijos (9 klausimai), apimantys gydymo grafikus, naudojamų antihelmintikų vartojimo dažnumą ir dozes, taip pat naujai įvežtų gyvulių karantino praktiką (1 priedas).

2.3. Ūkių atranka

Avių ūkiai buvo atrenkami iš Lietuvos avių augintojų asociacijos narių sąrašo, kuriame tyrimo metu buvo 193 registruoti ūkiai. Su visais sąraše esančiais avių augintojais buvo susisiekta telefonu sąrašo tvarka ir jie buvo pakviesti dalyvauti tyrime. Vienintelis būtinas atrankos kriterijus buvo reikalavimas, kad ūkyje būtų laikoma ne mažiau kaip 15 avių, norint užtikrinti reprezentatyvią aktyviai valdomų bandų imtį. Prieš mėginių ėmimą ūkininkų buvo klausiama, ar per pastarąsias 8 savaites jų ūkiuose buvo naudoti antihelmintiniai preparatai. Ši informacija buvo renkama siekiant sumažinti galimą neseniai atlikto gydymo įtaką išmatų kiaušinėlių skaičiaus ir *in vitro* atsparumo tyrimų rezultatams. Į tyrimą buvo įtraukti 38 avių ūkiai, kurios sutiko dalyvauti ir atitiko atrankos kriterijus, o tai sudarė 19,7 proc. visų kviestų dalyvauti ūkių. Tyrime dalyvavę ūkiai buvo išsidėstę visose dešimtyje Lietuvos apskričių, taip užtikrinant platų geografinį šalies avių sektoriaus atstovavimą. Imties dydis buvo nustatytas remiantis praktiniu įgyvendinamumu ir panašių tyrimų rezultatais [24, 80]. Siekiant išvengti atrankos šališkumo, ūkiai buvo atrenkami paeiliui iš viso Lietuvos avių augintojų asociacijos narių sąrašo, neatliekant išankstinės atrankos pagal įtariamą AA, nes neatsitiktinė atranka gali reikšmingai pervertinti AA paplitimą [5, 15].

2.4. Modifikuotas McMasterio tyrimas

Išmatų kiaušinėlių skaičius buvo nustatomas taikant modifikuotą McMaster metodą, kurio analitinis jautrumas siekė 20 kiaušinėlių grame išmatų (KSG). Mėginių rinkimas buvo vykdomas ganymo sezono metu. Kiekvienoje grupėje gyvuliai buvo atrenkami taikant patogumo atranką, norint įtraukti 10–15 gyvulių iš kiekvienos grupės. Siekiant užtikrinti reprezentatyvų bandos lygmens užsikrėtimo virškinamojo trakto nematodais įvertinimą, į tyrimą buvo įtrauktos vyresnės nei vienerių metų amžiaus avys, neatsižvelgiant į jų amžiaus kategoriją. Išmatų mėginiai buvo imami iš tiesiosios žarnos. Mėginiai buvo laikomi ir ištirti per 48 val. nuo surinkimo, naudojant modifikuotą McMasterio metodą. Kiekvienam tyrimui buvo pasverta 4 g išmatų ir jos sumaišytos su 56 ml vandentiekio vandens, o gauta suspensija buvo palikta pastovėti maždaug 30 min., kad stambesnės dalelės suminkštėtų. Tuomet išmatų suspensija buvo perfiltruota per arbatos sietelį arba dvigubą marlės sluoksnį į plastikinę kolbą, kad būtų pašalintos stambios dalelės. Iš filtruotos suspensijos 10 ml buvo perpilta į centrifugavimo mėgintuvėlį ir centrifuguota 7 min. 1200 aps./min. greičiu. Po centrifugavimo viršutinis sluoksnis buvo išpilamas, o likusios nuosėdos buvo pakartotinai išmaišytos cinko chlorido flotacijos tirpale (savitasis sunkis 1,40) iki 4 ml žymės. McMaster skaičiavimo kameros buvo užpildytos suspensija, vengiant oro burbuliukų. Leidus kamerai pastovėti apie 3 min., kad kiaušinėliai pakiltų į paviršių, šviesiniu mikroskopu buvo suskaičiuoti visi strongilidų (*T. circumcincta*, *Trichostrongylus* spp., *H. Contortus, Cooperia*), *Nematodirus* spp., *Strongyloides* spp. ir *Trichuris* spp. kiaušinėliai abiejų kamerų tinkleliuose. KSG išmatų buvo apskaičiuotas sudėjus abiejų kamerų kiaušinėlių skaičių ir padauginus gautą sumą iš 20.

2.5. *In vitro* antihelmintinio atsparumo nustatymas

2.5.1. Kiaušinėlių paruošimas *in vitro* tyrimui

Iš tų pačių 38 avių bandų, kurios dalyvavo KSG nustatymo tyrime, iš kiekvieno gyvulio buvo surinkta 6–10 g išmatų, taip gaunant bendrą maždaug 100–120 g mėginį. Išmatos buvo gerai sumaišytos, o trečdalis jų buvo perkeltas į 250 ml talpą, kuri buvo užpildyta maždaug iki dviejų trečdalių vandentiekio vandeniu ir sandariai uždaryta iki tolimesnio apdorojimo. Nematodų kiaušinėliai buvo išskiriami taikant nuoseklų plovimo metodą naudojant skirtingų diametrų laboratorinius sietus, kurių akutės buvo 250, 100 ir 20 µm. Ant 20 µm sieto likusi medžiaga buvo nuplauta į stiklinį indą, palikta nusistovėti 15–20 min., o viršutinis sluoksnis atsargiai pašalintas. Likusios nuosėdos buvo perkeltos į mėgintuvėlius ir centrifuguotos 2 min. 1500 aps./min. greičiu. Viršutinis sluoksnis buvo išpilamas, o cukraus tirpalas buvo pilamas tol, kol susiformavo meniskas, po to buvo atliktas antrasis centrifugavimo etapas. Prie dangtelių prilipę kiaušinėliai buvo nuplauti distiliuotu vandeniu į švarų indą. Gauta kiaušinėlių suspensija dar kartą nuplauta dejonizuotu vandeniu, kad būtų pašalinti cukraus likučiai. Tada mikropipete buvo paimta suspensija ir atliktas KSG tyrimas. Norint užtikrinti tolesnių tyrimų standartizavimą suspensija buvo atskiesta iki tokios koncentracijos, kad 10 µl sudarytų maždaug 100 kiaušinėlių [24, 80].

2.5.2. Mielių ekstrakto paruošimas *in vitro* LVMAT

Mielių ekstrakto tirpalas buvo paruoštas ištirpinant 1 g mielių ekstrakto 90 ml 0,85 proc. NaCl tirpalo. Gauta suspensija buvo sterilizuota autoklave 121 °C temperatūroje, esant 1,0 atm slėgiui, 20 min. Po autoklavavimo į 27 ml mielių ekstrakto tirpalo buvo įpilta 3 ml koncentruoto Earle'o subalansuoto druskos tirpalo. Paruoštas mišinys buvo padalytas į porcijas ir laikytas užšaldytas keletą mėnesių iki naudojimo. 10× Earle'o tirpalo sudėtis (g/100 ml) buvo tokia: KCl 0,04, $MgSO_4$ 0,2, NaCl 6,68, $NaHCO_3$ 2,2, NaH_2PO_4 0,14 ir $CaCl_2$ 0,02.

2.5.3. Lervų vystymosi mikro agare tyrimas

LVMAT buvo atliktas pagal Coles ir kt. [19] aprašytą protokolą. Tyrimai buvo atliekami 96-duobučių mikrotitravimo plokštelėse. Ivermektino aglikono (IVM-Ag) ir tiabendazolio (TBZ) pagrindiniai tirpalai buvo paruošti dvigubai serijiniu skiedimu dimetilsulfokside (DMSO), o LEV buvo skiedžiamas dejonizuotu vandeniu. TBZ buvo naudojamas kaip pavyzdinis junginys BZ klasės atsparumo tyrimams, o IVM-Ag – kaip pavyzdinis junginys IVM klasės atsparumo tyrimams. Buvo tiriama dvylika koncentracijų: 0,084–173,6 ng/ml IVM-Ag, 0,0006–1,28 µg/ml TBZ ir 0,0156–32 µg/ml LEV. Kiekvienai koncentracijai 12 µl vaisto tirpalo buvo įpilta į 150 µl 2 proc. Bacto agaro (Difco, JAV), o gautas mišinys buvo paliktas sukietėti 4 °C temperatūroje 5 min.. Norint išvengti grybelinės taršos, 10 µl kiaušinėlių suspensijos (maždaug 50 kiaušinėlių vienoje duobutėje) 0,3 mg/ml amfotericino B (Sigma-Aldrich, Vokietija) tirpale buvo sumaišyta su 10 µl mielių ekstrakto ir užpilta ant agaro. Mielių ekstraktas buvo paruoštas pagal Hubert ir Kerboeuf metodą: 1 g mielių ekstrakto buvo ištirpinta 90 ml 0,85 proc. NaCl tirpalo, suspensija autoklavuota 20 min. 121 °C temperatūroje, o tuomet 27 ml šio tirpalo papildyta 3 ml 10 kartų koncentruoto Earle'o tirpalo. Kontrolinėse duobutėse galutinė DMSO koncentracija buvo 1,3 proc. Plokštelės buvo inkubuojamos 7 dienas 27 °C temperatūroje. Vystymasis buvo sustabdomas į kiekvieną duobutę įlašinus Lugolio tirpalo. Neišsiritusių kiaušinėlių ir L1, L2 bei L3 stadijų lervų proporcijos buvo vertinamos naudojant stereomikroskopą [16, 24].

LVMAT duomenys buvo analizuojami taikant ribinių (diskriminacinių) koncentracijų metodą. Ūkiai buvo klasifikuojami kaip atsparūs, jei L3 stadijos lervos buvo aptiktos esant šioms ribinėms koncentracijoms: 21,6 ng/ml IVM-Ag, 0,04 µg/ml TBZ ir 2 µg/ml LEV [16, 24, 76, 80, 109]. L3 stadijos lervos, esant šioms ribinėms koncentracijoms, buvo atskirtos ir identifikuotos remiantis morfologiniais požymiais, naudojant stereomikroskopą [50].

2.6. Statistika ir duomenų analizė

2.6.1. Bedrosios statistinės proceduros

Duomenų analizė buvo atlikta naudojant programą *Microsoft Excel 2019* ir *IBM SPSS Statistics, version 29.0* (IBM Corp., Armonk, NY, USA; 2022). Duomenims apibendrinti ir ūkių lygmens kintamiesiems charakterizuoti buvo taikyta aprašomoji statistika. Tęstiniai kintamieji buvo aprašomi pateikiant vidurkius, medianas, tarpkvartilinius pločius ir reikšmių intervalus. Kategoriniai kintamieji buvo išreiškiami proporcijomis su 95 proc. pasikliautiniais intervalais (PI), siekiant įvertinti apskaičiuotų rodiklių tikslumą. Proporcijų PI buvo apskaičiuoti taikant Clopper–Pearson metodą, pagrįstą vienos imties binominiu skirstiniu, o binominių proporcijų palyginimams buvo naudojami Wilsono pasikliautiniai intervalai.

Sąsajos tarp kategorinių kintamųjų buvo vertinamos taikant Pearsono χ^2 kriterijų. Kai tikėtinas stebėjimų skaičius lentelės langelyje buvo mažesnis nei penki, statistinių išvadų patikimumui užtikrinti buvo taikytas tikslusis Fišerio kriterijus. Šis metodas taip pat buvo naudojamas analizuojant antihelmintinį atsparumą (įskaitant daugiavaistį atsparumą, DA), kai atsparumo statusas (atspari arba jautri banda) buvo lyginamas su ūkio lygmens veiksniais, tokiais kaip avių veislių grupė, naudojamų antihelmintikų klasės, importuotų avių buvimas bei gyvulių tankio kategorija. Tęstinių kintamųjų, įskaitant vidutinį gydymų dažnį, palyginimai tarp tradicinių ir ekologinių ūkių buvo atliekami naudojant nepriklausomų imčių Stjudento t kriterijų arba Manno-Whitney U kriterijų, priklausomai nuo duomenų pasiskirstymo. Statistinio reikšmingumo lygmuo buvo nustatytas, kai $p < 0,05$.

Gyvulių tankis (avių skaičius hektare) buvo apskaičiuotas bendrą avių skaičių padalijus iš deklaruoto ganyklų ploto ir buvo naudojamas kaip ganymo intensyvumo rodiklis ūkio lygmeniu. Analizės tikslais šis rodiklis buvo suskirstytas į tris kategorijas: mažas (< 3 avys/ha), vidutinis (3–7 avys/ha) ir didelis (> 7 avys/ha).

2.6.2. Kiaušinėlių skaičiaus išmatose ir *in vitro* tyrimų rezultatų analizė

Išsivysčiusių L3 lervų dalis esant ribinėms koncentracijoms buvo naudojama atsparumo laipsniui klasifikuoti kaip lengvam (< 10 proc.), vidutiniam (10–50 proc.) arba sunkiam (> 50 proc.). Ši klasifikacija atspindi tą lervų dalį, kurios išgyveno ir užbaigė vystymąsi nepaisant poveikio vaisto koncentracijai, mirtinai veikiančiai jautrias populiacijas – didesnė išgyvenusių L3 lervų dalis rodo didesnį atsparių individų dažnį nematodų populiacijoje. Todėl šis metodas leido ne tik nustatyti atsparumą (yra arba nėra), bet ir kiekybiškai įvertinti atsparumo intensyvumą kiekviename ūkyje.

Be to, buvo analizuojami KSG duomenys, gauti taikant McMasterio metodą, siekiant apibūdinti virškinamojo trakto nematodų infekcijų intensyvumą ir pasiskirstymą tiek individualiu, tiek bandos lygiu. Kiekvienam mėginiui buvo nustatytas KSG strongilidams, *Strongyloides* spp., *Nematodirus* spp. ir *Trichuris* spp., o aprašomoji statistika (minimumas, maksimumas, vidurkis, mediana, standartinis nuokrypis ir teigiamų mėginių procentinė dalis) buvo apskaičiuota visiems tirtiems gyvuliams. Kadangi KSG duomenys buvo labai asimetriški, infekcijos intensyvumas bandos lygiu buvo apibendrintas naudojant kiekvienos bandos strongilidų KSG medianą, o ne vidurkį, o tarpkvartilinis intervalas (IQR) buvo naudojamas apibūdinti skirtumus tarp bandų.

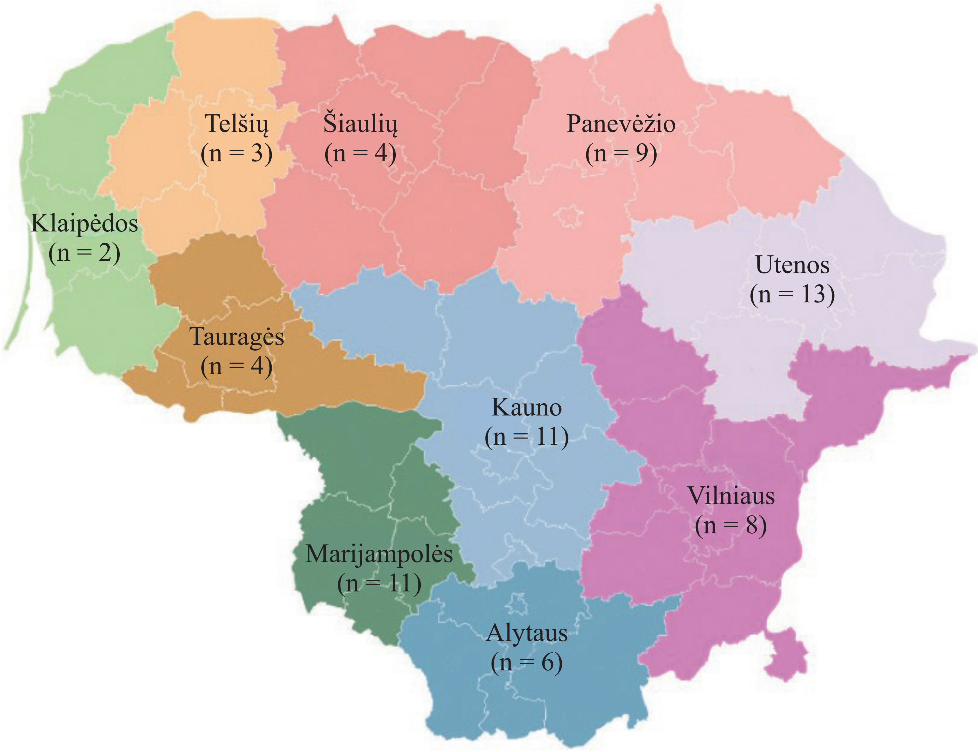
Bandos buvo suskirstytos pagal LVMAT nustatytą AA statusą į atsparias ir jautrias, o bandos lygio strongilidų KSG medianos vertės buvo palygintos tarp BZ ir IVM atsparių bei jautrių grupių. Grafiniam duomenų pateikimui buvo sudarytos dėžutės diagramos, vaizduojančios bandos lygio strongilidų KSG medianą pagal atsparumą BZ ir IVM. Bandos lygio strongilidų KSG medianos taip pat buvo palygintos tarp DA ir ne DA bandų naudojant neparametrinį Mann–Whitney U testą.

3. REZULTATAI

3.1. Apklausa dėl Lietuvos avių ūkiuose taikomų VTN kontrolės praktikų

3.1.1. Apklaustų ūkių bendrosios charakteristikos

Iš viso apklausoje dalyvavo 71 avių ūkis (71 / 193), o bendras atsako dažnis siekė 36,8 proc. (95 proc. PI: 29,9–44,1 proc.). Didžiausia respondentų dalis buvo Utenos apskrityje (13 / 71; 18,3 proc.) ir Kauno bei Marijampolės apskrityse (11 / 71; po 15,5 proc.) (3.1.1.1 pav.). Tarp apklaustų ūkių 60,6 proc. buvo oficialiai patvirtinti kaip ekologiniai ūkiai (43 / 71; 95 proc. PI: 48,8–71,5 proc.), o 39,4 proc. taikė tradicinius ūkininkavimo metodus (28 / 71; 95 proc. PI: 28,5–51,2 proc.). Vidutinis bandos dydis buvo 184,5 avys (intervalas: 10–1600 avys).



3.1.1.1 pav. *Apklaustų avių ūkių geografinis pasiskirstymas dešimtyje Lietuvos apskričių*

Rotacinis ganymas buvo taikomas 36,6 proc. ūkių (26 / 71; 95 proc. PI: 25,6–48,7 proc.), o likusiuose 63,4 proc. ūkių (45 / 71; 95 proc. PI: 51,3–74,4 proc.) avys visą ganymo sezoną buvo laikomos vienoje ganykloje. Vidutinis ganyklų plotas ūkiui buvo 34,51 ha (intervalas: 1–200 ha), tai rodo didelę ūkių įvairovę. Atsižvelgiant į gyvulių tankumą, vidutinis avių skaičius ha buvo 7,4 (mediana 5,6; tarpketvirtinis intervalas 3,6–9,8), o reikšmės svyravo nuo 1,3 iki 31,7 avies ha. Remiantis šiais duomenimis, ūkiai buvo suskirstyti į tris ganymo intensyvumo kategorijas: mažas (< 3 avys/ha), vidutinis (3–7 avys/ha) ir didelis (> 7 avys/ha). Dauguma ūkių pateko į vidutinio intensyvumo kategoriją (28 / 71; 39,4 proc.; 95 proc. PI: 28,1–51,9 proc.), 21,1 proc. (15 / 71; 95 proc. PI: 12,5–32,4 proc.) sudarė mažo tankumo ūkiai, ir tokia pati dalis (28 / 71; 39,4 proc.; 95 proc. PI: 28,1–51,9 proc.) – didelio tankumo ūkiai.

Lietuvos juodgalvių avių veislė tirtuose ūkiuose buvo dominuojanti ir sudarė 46,5 proc. (33 / 71); 95 proc. PI: 34,9–58,3 proc.). Kitos veislės ūkiuose pasiskirstė taip: mišrūnės – 18,3 proc., (13 / 71), merinosai – 5,6 proc., (4 / 71) ir sufolkai – 5,6 proc., (4 / 71). Ganymas dažniausiai prasidėdavo gegužės mėn., n = 34 / 71; 47,9 proc., rečiau balandžio, n = 30 / 71; 42,3 proc. ir 9,9 proc. kovo mėn., 7 bandose iš 71. Ganiava dažniausiai baigdavosi lapkritį (54 / 71; 76,1 proc.). Vienas ūkis ganymą užbaigdavo anksčiau – spalį (1,4 proc.). Tačiau dalis ūkių (22,5 proc.) ganymo laikotarpį pratęsdavo net iki gruodžio (16 / 71). Dauguma ūkių (87,3 proc.; 62 / 71; 95 proc. PI: 77,9–93,9 proc.) nelaikė kitų ūkinių gyvūnų rūšių. Tarp ūkių, kurie laikė ir kitus gyvūnus, 8,5 proc. kartu laikė ožkas (6 / 71), 2,8 proc. – galvijus (2 / 71), o 1,4 proc. – arklius (1 / 71), tai rodo, kad mišrios (keleto rūšių) ganymo sistemos tarp apklaustų ūkių buvo gana retos. Vandens tiekimo praktikos buvo įvairios: 67,6 proc. ūkių (n = 48 / 71; 95 proc. PI: 55,3–78,2 proc.) pagrindinis vandens šaltinis buvo šachtinių šulinių vanduo, o likusiuose ūkiuose buvo naudojami natūralūs ir kiti ūkyje prieinami vandens šaltiniai.

3.1.2. Ūkiuose tikomų VTN kontrolės strategijos

Beveik pusė ūkininkų, spręsdami, ar bandoje yra VTN infekcija, rėmėsi savo ankstesne patirtimi (40,9 proc.; n = 27), o viduriavimą nurodė kaip antrą pagal dažnumą kriterijų. Viduriavimo pasireiškimą, kaip VTN diagnostinį kriterijų, paminėjo maždaug ketvirtadalis respondentų (3.1.2.1 lentelė). Objektyvūs diagnostikos metodai, pavyzdžiui, išmatų tyrimas, buvo taikomi rečiau (19,7 proc.; n = 13), o veterinarijos gydytojų rekomendacijomis rėmėsi tik nedidelė dalis ūkių – mažiau nei 10 proc. (n = 4) respondentų. Tik nedidelė dalis ūkių (n = 5) nenurodė jokio aiškaus antihelmintikų pasirinkimo sprendimo kriterijaus.

3.1.2.1 lentelė. *Kriterijai, darantys įtaką ūkininkų sprendimams dėl virškinamojo trakto nematodų infekcijos vertinimo (n = 66)*

Kriterijus	n	proc.	95 proc. PI
Ankstesnė patirtis	27	40,9	29,9–53,0
Viduriavimo pasireiškimas	15	22,7	14,3–34,2
Išmatų tyrimas	13	19,7	11,9–30,8
Veterinarijos gydytojo rekomendacijos	4	6,1	2,4–14,6

Dauguma ūkių antihelmintinius preparatus naudojo prieš išginant avis į ganyklą (67,2 proc., n = 43), tuo tarpu gydymas prieš suvarymą į tvartą arba tvartinio laikotarpio metu buvo taikomas tik maždaug 15,6 proc., (n = 10) (3.1.2 lentelė). Gydymas pavasarį po išginimo į ganyklą ir prieš kergimą ar ėriavimąsi buvo taikomas dar rečiau, atitinkamai 7,8 proc., (n = 5) ir 4,7 proc., (n = 3), o tik pavieniai ūkiai nurodė gydantys avis perkeliant jas į naują ganyklą ar kelis kartus per ganymo ciklą (iš viso < 5 proc., n = 3). Be to, 8,5 proc. ūkių (n = 6) nurodė iš viso nenaudojantys antihelmintinių preparatų (3.1.2.2 lentelė).

3.1.2.2 lentelė. *Antihelmintinio gydymo laiko analizė avių ūkiuose*

Anthelmintikų skyrimo laikas	n / iš viso	proc.	95 proc. PI
Prieš išgenant į ganyklą	43 / 64	67,2	54,9–78,0
Tvartiniu laikotarpiu	10 / 64	15,6	7,8–26,9
Pavasarį po išgenimo	5 / 64	7,8	2,6–17,3
Prieš kergimą ar ėriavimąsi	3 / 64	4,7	1,0–13,1
Perkeliant gyvulius į naują ganyklą	1 / 64	1,6	0,0–8,4
Keliais laikotarpiais per ganymo ciklą	2 / 64	3,1	0,4–10,8
Anthelmintikai nenaudojami	6 / 71	8,5	3,2–17,5

Iš viso 90,2 proc. avių ūkių (n = 64) nurodė naudojantys antihelmintinius preparatus VTN kontrolei. Vidutiniškai ėriukams (< 1 metų amžiaus) ir vyresnėms avims, apimančioms jaunas (1–2 metų amžiaus) ir suaugusias (> 2 metų amžiaus) avis, gydymas buvo taikomas maždaug 1,5 karto per metus (atitinkamai 1,54 ir 1,49 gydymo karto), o daugumoje ūkių antihelmintiniai preparatai buvo skiriami vieną arba du kartus per metus. Beveik pusė ūkių jaunas ir suaugusias avis gydė du kartus per metus (49,3 proc.; n = 35), panaši tendencija stebėta ir ėriukams (43,7 proc.; n = 31), tuo tarpu nemaža dalis bandų buvo gydoma tik kartą per metus (38,0 proc.; n = 27 abiem grupėms). Intensyvesnės gydymo schemos, kai antihelmintiniai preparatai buvo skiriami tris kartus per metus, buvo taikomi palyginti retai (4,2 proc. ūkių suagusių avių grupei ir 9,9 proc. – ėriukams), o 8,5 proc. ūkių (n = 6) nurodė visai negydantys nei vienos gyvulių grupės (3.1.2.3 lentelė).

3.1.2.3 lentelė. *Anthelmintikų skyrimo dažnis ėriukams ir suaugusioms avims (n = 71)*

Grupė	Gydymų skaičius per metus	n	proc.	95 proc. PI
Jaunos ir saugusios avys	1	27	38,0	26,8–50,3
	2	35	49,3	37,2–61,4
	3	3	4,2	0,9–11,9
	Negydo	6	8,5	3,2–17,5
Ėriukai	1	27	38,0	26,8–50,3
	2	31	43,7	31,9–56,0
	3	7	9,9	4,1–19,3
	Negydo	6	8,5	3,2–17,5

Tradiciniuose ūkiuose antihelmintiniai preparatai buvo naudojami dažniau nei ekologiniuose. Tradiciniuose ūkiuose suagusioms avims vidutiniškai buvo taikoma 1,71 gydymo karto per metus, tai yra maždaug 1,27 karto daugiau nei ekologiniuose ūkiuose (1,35 gydymo karto per metus; $p < 0,05$). Panaši tendencija nustatyta ir ėriukams – tradiciniuose ūkiuose jiems buvo skiriama 1,71 gydymo karto per metus, palyginti su 1,44 gydymo karto per metus ekologiniuose ūkiuose (1,19 karto daugiau; $p < 0,05$).

Dauguma ūkių (45,1 proc.; n = 32) antihelmintikų dozes nustatė pagal vizualiai įvertintą gyvulio svorį. Dar 35,2 proc. (n = 25) ūkių dozę apskaičiavo pasverdami vidutinio dydžio avį ir gautą svorį taikydami visai gyvulių grupei. Tiksliausius svoriu pagrįstus metodus, kai sveriamas stambiausias bandos gyvulys arba kiekviena avis atskirai, taikė mažiau nei 10 proc. ūkių (8,4 proc.; n = 6). Maždaug vienas iš dešimties ūkių (11,3 proc.; n = 8) nurodė apskritai nenaudojantys antihelmintinių preparatų (3.1.2.4 lentelė).

3.1.2.4 lentelė. *Anthelmintikų dozės parinkimo metodai tirtuose avių ūkiuose (n = 71)*

Dozės nustatymo būdas	n	proc.	95 proc. PI
Vizualus svorio įvertinimas	32	45,1	33,6–56,9
Vidutinio dydžio avies svėrimas	25	35,2	24,6–47,2
Didžiausios avies svėrimas	4	5,6	1,5–13,6
Kiekvieno gyvulio svėrimas	2	2,8	0,3–9,7
Gydymas nenaudojamas	8	11,3	5,0–21,0

ML, vartoti atskirai arba deriniuose su kitomis antihelmintinių preparatų klasėmis, buvo pagrindinė naudojama vaistų grupė – juos naudojo beveik trys ketvirtadaliai ūkių (74,6 proc.; n = 53). Priešingai, BZ buvo naudojami kiek daugiau nei trečdalyje ūkių (35,2 proc.; 25 / 71), o LEV buvo skiriamas gerokai rečiau – tik 12,7 proc. ūkių (9 / 71) tiek kaip vienintelė, tiek kaip kelių klasių derinio sudėtinė dalis. Rotaciją tarp BZ ir ML aiškiai nurodė 16,9 proc. ūkių (12 / 71), tai rodo, kad tik mažesnė dalis ūkininkų aktyviai rotuoja vaistų klases (3.1.2.5 lentelė).

3.1.2.5 lentelė. *Anthelmintikų klasių naudojimo ir antihelmintikų rotacijos strategijos apklaustuose avių ūkiuose (n = 71)*

Antihelmintikų naudojimo modelis	n	proc.	95 proc. PI
ML	36	50,7	39,4–62,0
LEV	4	5,6	1,4–11,3
BZ	8	11,3	4,2–19,7
BZ + ML	12	16,9	8,5–25,4
BZ + ML + LEV	5	7,0	1,4–14,1
Gydymas netaikytas	6	8,5	2,8–15,5

Nustatyta, kad 12,7 proc. ūkininkų (9 / 71) importavo avis iš užsienio šalių. Vertinant visas naujai į bandas įvestas avis, nepriklausomai nuo to, ar jos buvo importuotos iš užsienio, ar įsigytos kituose Lietuvos ūkiuose, karantino priemonės buvo taikomos 45,1 proc. ūkių (32 / 71), o 54,9 proc. ūkių (39 / 71) karantino netaikė.

3.2. Virškinimo trakto nematodų paplitimas

Taikant McMaster metodą, buvo ištirta 400 avių išmatų mėginių iš 38 bandų. Strongilidų kiaušinėlių skaičius pasižymėjo labai didele įvairove – nuo labai žemo lygio iki labai didelio užsikrėtimo (> 8000 KSG), o mediana siekė 60 KSG, kas rodo, kad dauguma gyvulių turėjo nedidelį arba vidutinį užsikrėtimą strongilidais. Priešingai, *Nematodirus* spp. ir *Trichuris* spp. kiaušinėlių skaičius apskritai buvo labai mažas, su nulinėmis KSG medianomis ir tik pavieniais mėginiais, kuriuose nustatyti didesni kiekiai, o *Strongyloides* spp. didelės KSG vertės pasitaikė tik nedidelėje dalyje gyvulių. Populiacijos lygmeniu strongilidų kiaušinėliai buvo aptikti maždaug trijuose ketvirtadaliuose visų tirtų mėginių, tuo tarpu *Strongyloides* spp., *Nematodirus* spp. ir *Trichuris* spp. kiaušiniai nustatyti tik mažumoje mėginių, patvirtinant, kad strongilidai buvo dominuojantys virškinamojo trakto nematodai tirtose bandose (3.2.1 lentelė).

3.2.1 lentelė. *Virškinamojo trakto nematodų KSG rodikliai ir paplitimas tirtuose mėginiuose (n = 400 mėginių iš 38 bandų)*

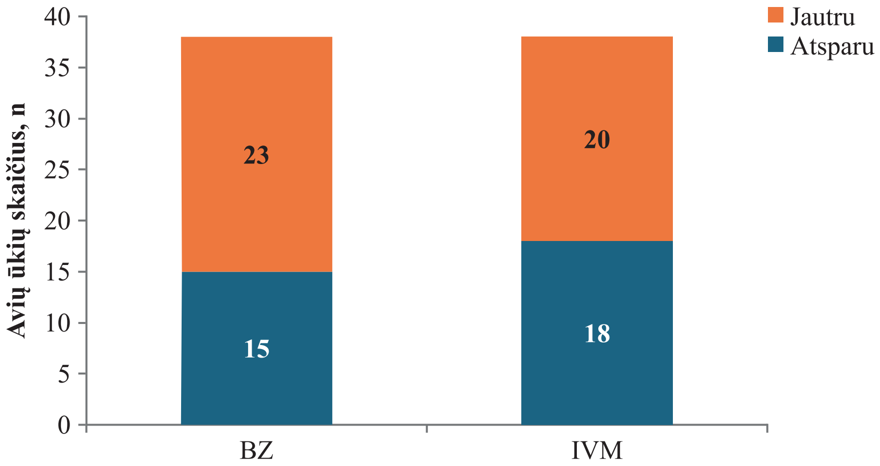
	Min.	Maks.	Vidurkis	Mediana	SN	Proc. teigiamų
Strongyloides spp.	0	760	18,77	0,0	58,29	30,0
Nematodirus spp.	0	220	3,45	0,0	18,97	5,5
Strongilidai	0	8120	268,40	60,0	839,57	75,2
Trichuris spp.	0	160	1,00	0,0	8,67	3,2

Bandos lygmeniu vertinant strongilidų kiaušinėlių skaičių (2 priedo lentelė), nustatyta didelė užsikrėtimo intensyvumo variacija tarp bandų. Bandų KSG medianos svyravo nuo 0 iki 710 KSG (mediana – 50,0 KSG; TKI 32,5–117,5 KSG), o bandų medianų vidurkis siekė 110,5 ± 151,1 KSG. Nedidelė dalis bandų pasižymėjo ypač dideliu užsikrėtimo intensyvumu – jų KSG medianos siekė 710 KSG (ID 1), 460 KSG (ID 2) ir 440 KSG (ID 3). Šiose bandose vidutinis strongilidų kiaušinėlių skaičius buvo didžiausias ir siekė iki 1970 KSG, o maksimalūs atskirų gyvulių rodikliai svyravo nuo 5400 iki 8120 KSG. Priešingai, penkiose bandose (ID 34–38) strongilidų KSG mediana buvo lygi nuliui. Šiose bandose taip pat nustatyti maži vidutiniai KSG rodikliai (13–36 KSG daugumoje bandų), o maksimalios reikšmės keturiose iš jų neviršijo 200 KSG.

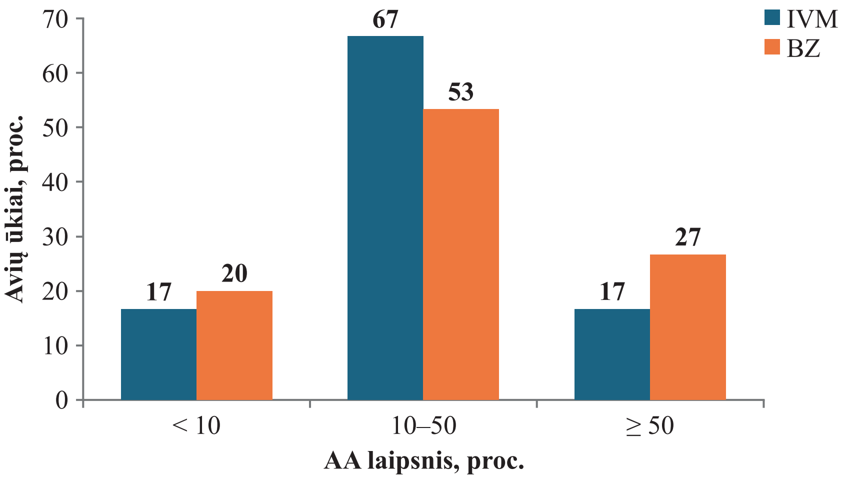
Mažiau paplitusioms nematodų rūšims bandų KSG mediana taip pat dažniausiai buvo nulinė, nors dalis gyvulių kai kuriose bandose buvo ir teigiami. Visose bandose strongilidų kiaušinėlius išskiriančių gyvulių dalis svyravo nuo 10 iki 100 proc., tuo tarpu *Strongyloides* teigiamų gyvulių dalis neviršijo 40 proc., o *Nematodirus* ir *Trichuris* teigiamų gyvulių dalis dažniausiai buvo dar mažesnė (2 priedų lentelė). Daugumoje bandų *Nematodirus*, *Strongyloides* ir *Trichuris* KSG mediana taip pat buvo nulis, tačiau procentiniai duomenys rodo, kad kai kuriose bandose iki trečdalio gyvulių išskyrė *Strongyloides* kiaušinėlius, o iki 10–20 proc. – *Nematodirus* ar *Trichuris* kiaušinėlius.

3.3. Atsparumo anthelmintikams paplitimas

LVMAT tyrimo rezultatai parodė, kad atsparumas bent vienai plataus veikimo spektro antihelmintikų klasei nustatytas daugiau nei pusėje tirtų ūkių (57,9 proc.; 22 / 38; 95 proc. PI: 40,8–73,3). L3 vystymasis esant ribinei IVM koncentracijai parodė IVM atsparumą 47,4 proc. ūkių (18 / 38; 95 proc. PI: 31,0–64,2), o BZ atsparumas nustatytas 39,5 proc. ūkių (15 / 38; 95 proc. PI: 24,0–56,6) (3.3.1 pav.). Tarp IVM atsparių ūkių vidutinio laipsnio atsparumas nustatytas 12 ūkių (12 / 18; 66,7 proc.), o lengvas ir stiprus atsparumas po 3 ūkius kiekvienoje kategorijoje (3 / 18; po 16,7 proc.). Tarp BZ atsparių ūkių aštuoni (8 / 15; 53,3 proc.) pasižymėjo vidutinio laipsnio atsparumu, keturi (4 / 15; 26,7 proc.) – stipriu atsparumu, o trys (3 / 15; 20,0 proc.) – lengvu atsparumu (3.3.2 pav.). LEV atsparumo nė vienoje iš tirtų bandų nenustatyta. Pažymėtina, kad BZ atsparumas nustatytas keliuose ūkiuose, kuriuose BZ preparatai nebuvo naudoti (8 / 15 BZ atsparių ūkių), o IVM atsparumas nustatytas viename ūkyje, kuriame IVM naudojimas nebuvo nurodytas.



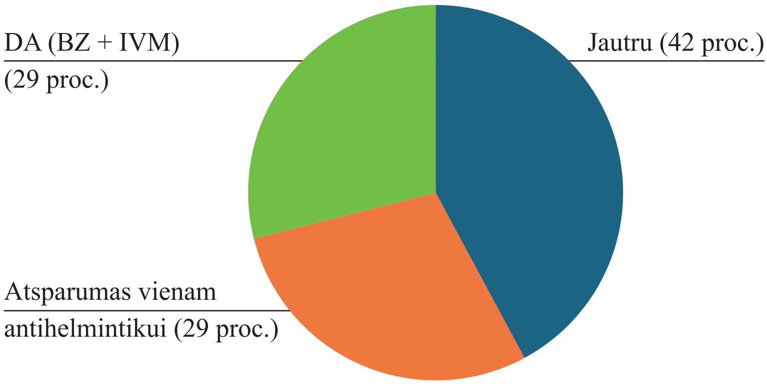
3.3.1 pav. *BZ ir IVM atsparumo antihelmintikams paplitimas tirtose avių bandose*



3.3.2 pav. *Avių ūkių pasiskirstymas pagal AA laipsnį IVM ir BZ atžvilgiu, nustatytą taikant LVMAT*

3.4 Atsparumo vienai antihelminikų klasei ir dauginio atsparumo paplitimas

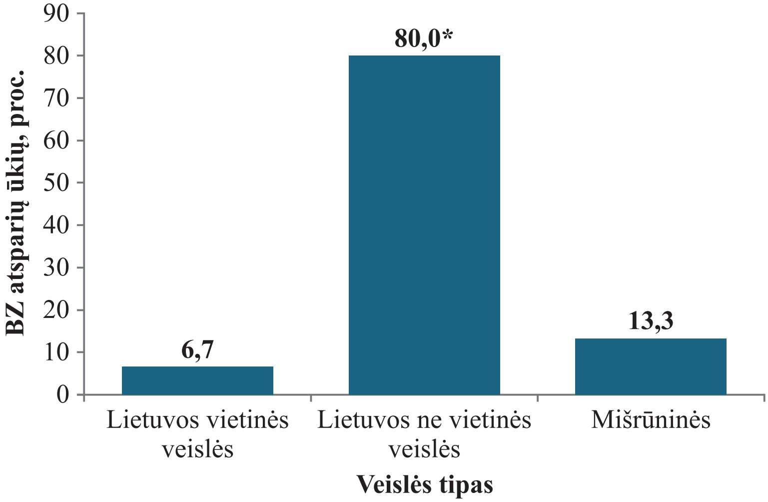
Iš 38 tirtų ūkių 16 nebuvo nustatytas atsparumas nė viena–m iš tirtų antihelmintinių preparatų. Atsparumas bent vienai vaistų klasei nustatytas 22 ūkiuose (57,9 proc.), o vienos klasės atsparumas ir DA, t. y. atsparumas tiek BZ, tiek IVM, buvo nustatyti vienodai dažnai – po 11 ūkių (28,9 proc.). Iš ūkių, kuriuose nustatytas vienos klasės atsparumas, dažniausiai buvo nustatytas atsparumas tik IVM (6 / 11 ūkių), rečiau – tik BZ (4 / 11). DA sudarė pusę visų atsparių bandų (11 / 22), o šiek tiek daugiau nei dviem penktadaliams ūkių išliko visiškas jautrumas visoms trims tirtoms antihelmintinių vaistų klasėms (3.4.1 pav.).



3.4.1 pav. *Avių ūkių dalis, kuriuose nenustatytas AA, nustatytas atsparumas vienam vaistui ir DA pagal LVMAT rezultatus (n = 38 ūkių)*

3.5. Sąsajos tarp ūkinių veiksnių ir atsparumo anthelmintikams

Atsparumas BZ buvo reikšmingai susijęs su avių veislės tipu (p = 0,001): BZ atsparumas dažniau nustatytas nevietinių veislių avių bandose, tuo tarpu Lietuvos vietinių ir mišrių veislių bandose jis buvo nustatomas gerokai rečiau (3.5.1 pav.). Priešingai, statistiškai reikšmingos sąsajos tarp veislės tipo ir atsparumo IVM nenustatyta, nors stebėta tendencija didesniam atsparumo paplitimui nevietinių veislių bandose (p = 0,06). Mišrių veislių bandose statistiškai reikšmingų sąsajų su atsparumu nei BZ, nei IVM nenustatyta (p > 0,05).



3.5.1 pav. *Avių veislės tipo ir BZ atsparumo sąsaja avių ūkiuose (*p = 0,001)*

Be to, BZ preparatų naudojimas buvo statistiškai reikšmingai susijęs su atsparumu BZ: dauguma BZ naudojančių ūkių buvo priskirti BZ atsparių ūkių grupei (81,8 proc.), palyginti su tik maždaug penktadaliu BZ nenaudojančių ūkių (22,2 proc.). Priešingai, IVM naudojimas nebuvo reikšmingai susijęs su atsparumu IVM, nes panašus atsparumo paplitimas nustatytas tiek ūkiuose, kurie naudojo IVM, tiek ūkiuose, kuriuose IVM nebuvo naudojamas. Tikėtina, kad tai galėjo nulemti nedidelis IVM nenaudojančių ūkių skaičius tyrimų grupėje. Metinis dehelmintizacijos dažnis taip pat nerodė reikšmingo ryšio su atsparumo intensyvumu nė vienam iš tirtų antihelmintinių preparatų: daugumoje ūkių vaistai buvo skiriami du kartus per metus, o dalis ūkių gydė vieną ar tris kartus per metus arba išvis negydė. Tarp ūkių, kuriuose buvo gauti teigiami LVMAT rezultatai, DA nustatytas 28,9 proc. bandų ir buvo statistiškai reikšmingai dažniau pasitaikantis ūkiuose, laikančiuose ne vietines avių veisles, palyginti su ūkiais, kuriuose laikomos tik vietinės veislės (p = 0,007) (3.5.1 lentelė).

3.5.1 lentelė. *Veiksnių, susijusių su antihelmintiniu atsparumu, analizė avių ūkiuose (n = 38)*

Veiksnys	Kategorija	Atsparūs ūkiai (proc.)	Statistinė sąsaja
BZ naudojimas ir BZ atsparumas	Naudoja	9 / 11 (81,8 proc.) BZ atsparūs	p = 0,001
	Nenaudoja	6 / 27 (22,2 proc.) BZ atsparūs	
IVM naudojimas ir IVM atsparumas	Naudoja	17 / 32 (53,1 proc.) IVM atsparūs	p > 0,05*
	Nenaudoja	1 / 5 (20,0 proc.) IVM atsparūs	
Metinis dehelmintizacijos dažnis	0 gydymų per metus	4 / 38 ūkių (10,5 proc.)	IVM: p = 0,103; BZ: p = 0,234
	1 gydymų per metus	8 / 38 ūkių (21,1 proc.)	
	2 gydymų per metus	23 / 38 ūkių (60,5 proc.)	
	3 gydymų per metus	3 / 38 ūkių (7,9 proc.)	
DA ir avių veislė	Vietinės avių veislės	2 / 20 (10,0 proc.)	
	Ne vietinės avių veislės	9 / 18 (50,0 proc.)	p = 0,007

* Skirtumas nebuvo statistiškai reikšmingas (p > 0,05), tikėtina, dėl nedidelio ūkių, nenaudojančių IVM (n = 5), skaičiaus.

3.6. Gydymo sprendimų kriterijų, dozavimo ir anthelmintinio atsparumo sąsajos

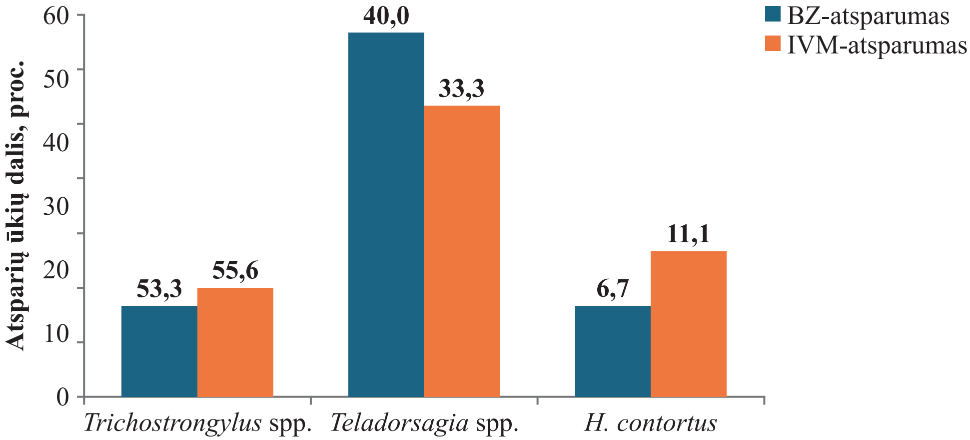
Gydymo sprendimų priėmimo kriterijai ūkiuose nebuvo statistiškai reikšmingai susiję su atsparumo BZ (p = 0,695), IVM (p = 0,983) ar DA (p = 0,829) pasireiškimu. Analogiškai, statistiškai reikšmingos sąsajos tarp antihelmintikų dozės apskaičiavimo metodo ir atsparumo BZ (p = 0,149), IVM (p = 0,218) ar DA (p = 0,078) taip pat nenustatyta (3.6.1 lentelė).

3.6.1 lentelė. *Gydymo sprendimų kriterijų ir dozavimo praktikos sąsajos su anthelmintiniu atsparumu 38 avių ūkiuose*

Kintamasis	Kategorija	Atsparumas BZ, n (%)	Atsparumas IVM, n (%)	DA, n (%)
Gydymo sprendimo priėmimo kriterijus	Laboratoriniai tyrimai	2 / 6 (33,3)	3 / 6 (50,0)	1 / 6 (16,7)
	Klinikiniai požymiai	6 / 12 (50,0)	6 / 12 (50,0)	4 / 12 (33,3)
	Ankstesnė patirtis / kitų patarimai	6 / 15 (40,0)	7 / 15 (46.7)	5 / 15 (33.3)
	Negydoma	1 / 5 (20,0)	2 / 5 (40,0)	1 / 5 (20,0)
Dozavimo metodas	Obijektyvus dozavimas (didžiausios avies ar kiekvienos avies svėrimas)	2 / 2 (100,0)	2 / 2 (100,0)	2 / 2 (100,0)
	Subjektyvus dozavimas (vizualus svorio įvertinimas, vidutinio dydžio avies svėrimas)	13 / 36 (36,1)	16 / 36 (44,4)	9 / 36 (25,0)

3.7. Atsparių nematodų genčių identifikavimas

L3 lervų diferenciacija ribinėse koncentracijose leido nustatyti nematodų gentis, susijusias su atsparumu antihelmintikams tirtuose ūkiuose. Tarp BZ atsparių ūkių *Trichostrongylus* spp. nustatyta 8 ūkiuose (53,3 proc.; 95 proc. PI: 26,6–78,7 proc.), *Teladorsagia* spp. – 6 ūkiuose (40,0 proc.; 95 proc. PI: 16,3–67,7 proc.), o *H. contortus* – 1 ūkyje (6,7 proc.; 95 proc. PI: 0,2–31,9 proc.), kas rodo, kad atsparumas BZ daugiausia buvo susijęs su *Trichostrongylus* ir *Teladorsagia* infekcijomis. IVM atspariuose ūkiuose *Trichostrongylus* spp. nustatyta 10 ūkių (55,6 proc.; 95 proc. PI: 30,8–78,5 proc.), *Teladorsagia* spp. – 6 ūkiuose (33,3 proc.; 95 proc. PI: 13,3–59,0 proc.), o *H. contortus* – 2 ūkiuose (11,1 proc.; 95 proc. PI: 1,4–34,7 proc.), o kitų nematodų genčių ribinėse koncentracijose nenustatyta (3.6.6 pav.). DA buvo patvirtintas viename ūkyje, kuriame aptikta *H. contortus*, o likusiais DA atvejais dominavo *Trichostrongylus* spp. (5 / 11 ūkių; 45,5 proc.; 95 proc. PI: 16,7–76,6 proc.) ir *Teladorsagia* spp. (5 / 11 ūkių; 45,5 proc.; 95 proc. PI: 16,7–76,6 proc.) (3.7.1 pav.).

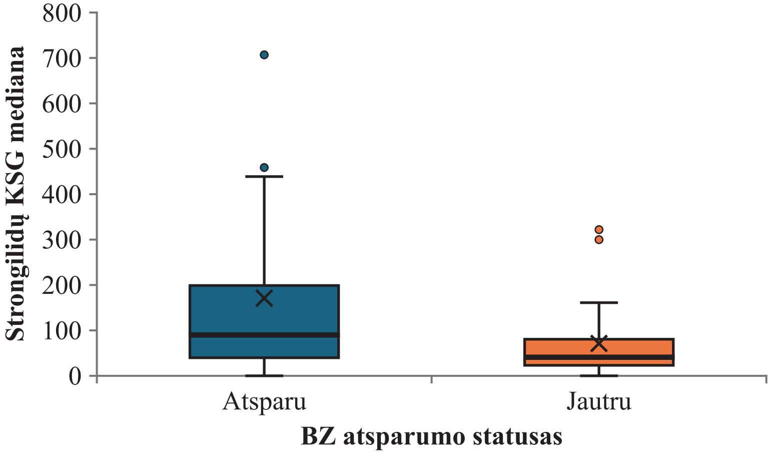


3.7.1 pav. *Atspariuose ūkiuose nustatytos nematodų rūšys*

3.8. Sąsaja tarp kiaušinėlių skaičiaus išmatose ir atsparumo antihelmintikams

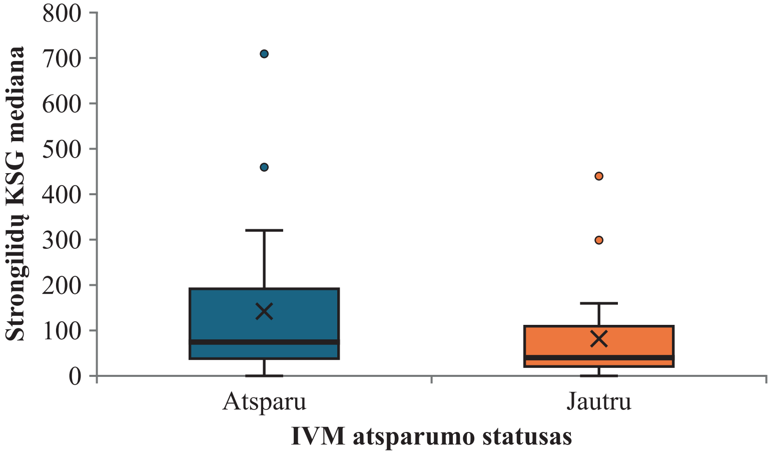
Norint įvertinti, ar bandos užsikrėtimo lygmens intensyvumas susijęs su AA, McMaster metodo rezultatai buvo palyginti tarp bandų, klasifikuotų kaip atsparios arba jautrios pagal *in vitro* tyrimus.

BZ atspariose bandose (n = 15) strongilidų kiaušinėlių koncentracijos mediana buvo didesnė nei BZ jautriose bandose (n = 23): strongilidų KSG mediana BZ atspariose bandose siekė 90 KSG (intervalas 0–710), palyginti su 40 KSG (intervalas 0–320) bandose, kuriose atsparumas BZ nenustatytas (3.8.1 pav.). Panašiai IVM atspariose bandose (n = 18) strongilidų KSG mediana buvo didesnė nei IVM jautriose bandose (n = 20) – atitinkamai 75 KSG (intervalas 0–710) ir 40 KSG (intervalas 0–440) (3.8.2 pav.).



3.8.1 pav. *Bandos lygmens strongilidų KSG mediana pagal BZ atsparumo statusą 38 avių bandose Lietuvoje*

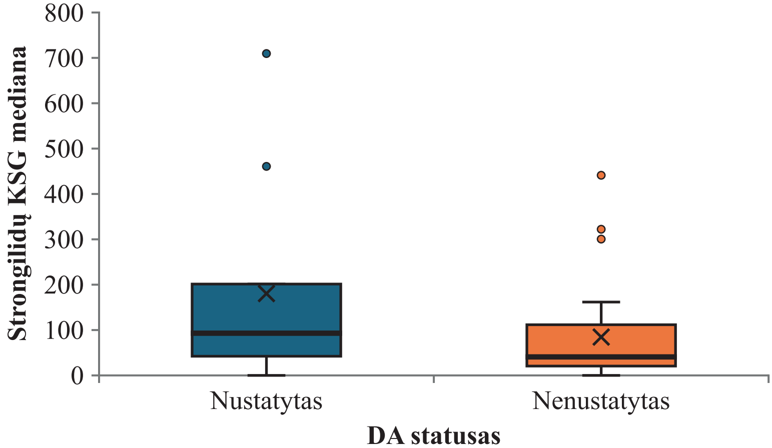
Pastaba: horizontali linija dėžutėje žymi medianą, „×“ – vidurkį, o taškai – išskirtines reikšmes (angl. *outliers*).



3.8.2 pav. *Bandos lygmens strongilidų KSG mediana pagal IVM atsparumo statusą 38 avių bandose Lietuvoje*

Pastaba: horizontali linija dėžutėje žymi medianą, „×“ – vidurkį, o taškai – išskirtines reikšmes (angl. *outliers*).

Bandose, kuriose nustatytas DA (n = 11), bandos lygmens strongilidų kiaušinėlių skaičiaus išmatose mediana buvo didesnė nei bandose kuriose DA nenustatytas (atitinkamai 90 KSG, IQR 50–195, palyginti su 40 KSG, IQR 25–95; Mano–Vitnio U = 201, p = 0,093), nors šis skirtumas nepasiekė statistinio reikšmingumo lygio.



3.8.3 pav. *Bandos lygmens strongilidų KSG medianos pagal daugialypio atsparumo statusą 38 Lietuvos avių ūkiuose*

Pastaba: horizontali linija dėžutėje žymi medianą, „×“ – vidurkį, o taškai – išskirtines reikšmes (angl. *outliers*).

IŠVADOS

1. Nustatyta, kad 45,1 proc. tirtų ūkių vaistų dozės parenkamos pagal vizualiai įvertintą gyvulio svorį, naujai įsigytoms avims karantino priemonės dažnai netaikomos – dauguma ūkininkų renkasi dehelmintizavimą be karantino (54,9 proc.), parazitologiniai išmatų tyrimai atliekami tik nedidelėje dalyje ūkių (19,7 proc.).
2. Virškinimo trakto strongilidų infekcijos buvo paplitusios visose tirtose avių bandose ir dominavo tarp nustatytų nematodų pagal paplitimą bei infekcijos intensyvumą. *Strongyloides* spp., *Nematodirus* spp. ir *Trichuris* spp. paplitimas buvo nedidelis.
3. Atsparumas LEV nenustatytas nė vienoje tirtoje bandoje. Daugiau nei pusėje bandų buvo nustatytas atsparumas IVM (47,4 proc.) ir (arba) BZ (39,5 proc.), kuris dažniausiai buvo vidutinio laipsnio. DA nustatytas 28,9 proc. bandų. Atspariose nematodų populiacijose dominavo *Trichostrongylus* spp., *Teladorsagia* spp. ir *H. contortus*.

4. BZ naudojimas nevietinių veislių avių bandose reikšmingai susijęs su dažnesniu atsparumo šios klasės preparatams nustatymu ($p = 0,001$). DA ir atsparumas BZ buvo dažniau nustatytas nevietinių veislių avių bandose (atitinkamai $p = 0,007$ ir $p = 0,001$). Gydymo sprendimo kriterijus ir antihelmintikų dozės apskaičiavimo būdas su atsparumo BZ, IVM ar DA pasireiškimu buvo nesusiję ($p > 0,05$).

PRAKTINĖS REKOMENDACIJOS

1. Kiaušinėlių skaičius avių išmatose turėtų būti nustatomas bent vieną–du kartus per ganymo sezoną, o gydymo sprendimai turėtų būti priimami remiantis šių tyrimų rezultatais.
2. Vaistų klasė turėtų būti parenkama atsižvelgiant į bandoje nustatytą antihelmintinį atsparumą bei koprologinių tyrimų rezultatus.
3. Antihelmintikų dozės turi būti apskaičiuojamos pagal sunkiausios grupėje avies svorį arba individualiai įvertinus kiekvieno gyvūno kūno svorį.
4. LEV rekomeduojama įtraukti į parazitų kontrolės programas bandose, kuriose patvirtintas atsparumas BZ arba IVM.
5. Visiems naujai įsigytiems gyvuliams turėtų būti taikomas karantinas, kurio metu atliekami koprologiniai tyrimai ir prireikus taikomas gydymas antihelmintikais prieš gyvulius išleidžiant į bendras ganyklas.

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PUBLICATIONS

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RESEARCH

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Anthelmintic resistance in small ruminants in the Nordic-Baltic region

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Abstract

Gastrointestinal nematodes (GIN) in small ruminants result in production losses, and consequently economic losses, and are an animal welfare problem in most countries in the Nordic-Baltic region. Intensive use of anthelmintics to control helminth infections has led to anthelmintic resistance (AR), which has become a major issue in many European countries. Several studies have been performed in countries in the Nordic-Baltic region (e.g. Denmark, Sweden, Norway and Lithuania) showing increasing/emerging levels of AR. The aim of this paper is to provide an overview of the problem of AR on sheep and goat farms in the Nordic-Baltic region. This region has a limited number of registered anthelmintics. However, researchers in this area have discovered some surprising findings, such as ivermectin (IVM) resistance on a farm that had never used IVM. In Sweden there is evidence of macrocyclic lactone (ML)-resistant *Haemonchus contortus* being introduced with sheep imported from the Netherlands. As elsewhere in the world, the livestock trade appears to be contributing to the spread of AR in the region and isolated cases of multidrug-resistant cases have also been reported. This is surprising given that the frequency of treatments here is much lower than in other countries where sheep production is economically more important. The prevailing nematodes are *Haemonchus*, *Teledorsagia* and *Trichostrongylus*, while on some farms *Haemonchus* is dominant and clinical haemonchosis has increasingly been observed in recent decades. The reasons for this are unclear, but are probably related to this parasite's propensity to rapidly develop drug resistance and a general lack of awareness of the problem, possibly in combination with global warming and the increased livestock trade within the EU. In addition, domestic interactions through contacts with wildlife ruminants, alpacas may also be a contributing factor for transmission of AR.

Keywords: Gastrointestinal nematodes, Goat, Northern Europe, Sheep

Background

Gastrointestinal nematode (GIN) infections remain the most prevalent and important parasitic disease in grazing ruminants worldwide, affecting the welfare and health of animals and resulting in significant economic losses, especially in the sheep and goat industry [1–3]. Infections with GIN may lead to diarrhoea and digestive problems, anaemia and even animal death but most commonly, infections are subclinical and result in an overall decrease in productivity [4–6].

From the early 1960s broad-spectrum anthelmintics administered routinely have helped to control GIN, but intensive and incorrect use of anthelmintics, under-dosing and continuous treatments with the same anthelmintics have led to nematode populations resistant to anthelmintic drugs [1, 7–9]. Anthelmintic resistance (AR) is a heritable genetic trait in worms to resist a normally effective dose of the anthelmintic, which gradually becomes widespread among the hosts [10]. Most on-farm screening studies are based on the faecal egg count reduction test (FECRT), sometimes

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in combination with in vitro tests, e.g. the egg hatch test (EHT) and the micro-agar larval development test (MALDT), or molecular tests. On some farms, there is not just resistance to a single drug, but to multiple or even all available anthelmintics. To date, this has mainly been documented on sheep farms in the UK [11–13], Germany [14], Switzerland [15] and Belgium [16]. The occurrence of AR, including multidrug resistance, seems to be increasing in the Nordic-Baltic region [17] as well, and this will complicate the control of GIN in small ruminants in general. Although the efficacy of different anthelmintics is evaluated occasionally in some countries, it should be emphasized that in most Nordic-Baltic countries, randomised FECRT surveys have not recently been carried out. This situation is seen as dangerous because regular monitoring programmes for detecting the development and spread of AR are important in order to detect the problem in due time and thus be able to take steps to prevent the nationwide spread of resistant strains.

The aim of this paper is to provide an overview of the problem of AR on sheep and goat farms in the Nordic-Baltic region. This region has a limited number of registered anthelmintics. However, researchers in this area have discovered some surprising findings, such as ivermectin (IVM) resistance on a farm that had never used IVM [18]. In Sweden there is evidence of macrocyclic lactone (ML)-resistant *Haemonchus contortus* being introduced with sheep imported from the Netherlands [19].

Search strategy

This overview is based on a search in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) using the terms "anthelmintic resistance or multidrug resistance, nematodes, goat, sheep, small ruminants". The titles and abstracts were evaluated, and articles referring to AR were obtained and assessed in detail. The authors' extensive experience with AR in small ruminants was used to critically evaluate the literature. Nematodes will be addressed at genus-level unless worm counts with proper identification of species have been performed.

Review

Intensive use of anthelmintics will inevitably lead to AR in GIN. There is widespread emergence of nematodes resistant to benzimidazoles (BZ), levamisole (LEV) and IVM and other ML around the world and it is expanding in many parts of Europe. After a brief overview of AR of GIN in the EU, a more detailed account of AR in the Nordic-Baltic region is given below.

Current AR situation in the EU

AR in parasites is now widespread throughout Europe and has increased over a 41-year period, although there are still gaps in knowledge in some regions, especially eastern Europe [20]. At the European level, resistance has developed not only in *Haemonchus,* but in *Teladorsagia, Trichostrongylus, Cooperia* and *Nematodirus* as well [20, 21]. In most countries, recent reports of AR mainly refer to cases of BZ resistance, with increasing numbers of cases of ML resistance, particularly to IVM [20, 22]. According to Vineer et al. [20], a meta-analysis of AR in sheep and goats in Europe since 2010 revealed average farm-level prevalences of resistance to BZ of 86%, to ML except moxidectin 52%, to LEV 48% and to moxidectin (MOX) 21%, excluding single-farm case reports. There have also been some reports of AR to doramectin in the Netherlands and to MOX in Switzerland and southern Germany [23, 24], as well as to monepantel (MOP) in the United Kingdom (UK) [25]. Multiple AR in sheep GIN has been detected in France, Greece and Italy [26]. Multidrug resistance has also been reported in the UK and Ireland and is an emerging problem in Scotland [12, 13, 27] and France [28].

The most widely used anthelmintics in small ruminants in Europe are BZ and IVM. AR to BZ has been found on up to 98% of sheep farms examined in the Czech Republic [29], 14% in Spain [30], 100% in the Slovak Republic [31] and 100% in Ireland [32]. In the UK, AR to BZ is 98% and in Scotland alone it is 64%. Furthermore, IVM and LEV are becoming ineffective in most parts of the UK [11–13, 25]. In Italy, AR and suspected resistance to BZ has been found on 40% of dairy goat farms and resistance to eprinomectin on 20% of these farms [33]. Meanwhile, in Belgium, AR to BZ, ML, closantel and MOP has been found on sheep farms [16] and in Poland, AR to BZ and to IVM has been detected on 89% and 76% of sheep farms respectively [34, 35].

AR in the Nordic-Baltic region

AR is an increasing problem in all Nordic countries and several studies on this have been carried out in Denmark, Sweden, Norway and Lithuania. The first study on resistance in sheep nematodes carried out anywhere in the region was undertaken in Denmark and published in 1991, and described resistance to LEV in *Teladorsagia* [36]. Later, Maingi et al. (1996) reported evidence of BZ, IVM and LEV resistance in trichostrongyle nematodes in Danish goat herds, and the occurrence of AR to the BZ drug thiabendazole (4/13 herds), LEV (7/11 herds) and IVM (4/7 herds) on Danish sheep farms using FECRT and EHT [18, 37]. A 2014 study indicated that AR to the most commonly used anthelmintics was widespread in goat herds throughout Denmark. *Haemonchus contortus* was the predominant species in around half of the farms pre-treatment. Resistance to one or more anthelmintics was detected in nine out of 10 herds (90%). In the FECRT, resistance to BZ (fenbendazole) was present in all three herds tested, and to IVM in eight herds (80%). Based on EHT, resistance to BZ (EC50 value > 0.1 µg/ml) was found in 8 of the 10 herds [38]. Peña-Espinoza et al. (2014) also described a case of AR to BZ and IVM on one of the largest organic small ruminant farms in Denmark, whereas MOX and LEV were highly effective in the FECRT. BZ resistance in *H. contortus* and *T. colubriformis* was confirmed by the controlled efficacy test and pyrosequencing. IVM eliminated 100% of *H. contortus* and reduced *T. colubriformis* worm counts by 84–92% [39].

In Sweden, AR in small ruminants used to be relatively low. In a survey on sheep farms carried out in the 1990s, BZ (febantel and fenbendazole) was implicated in resistance in *H. contortus*, whereas IVM was highly effective [40]. Also, in a more recent study, only 4% (2/45) of randomly selected sheep farms showed evidence of BZ (albendazole) resistance in *H. contortus* by FECRT [41]. However, the allele frequency of the position of the codon 200 TAC mutation in the β-tubulin gene was measured by pyrosequencing and showed that BZ resistance was probably more widespread than that indicated by the FECRT. Parasitological diagnosis of "clinical" resistance to IVM was also found in two flocks. However, both the pre-treatment faecal egg counts and the reduction in these were low, and only three lambs had between 100 and 450 eggs/g faeces after treatment [41]. However, there has been evidence recently of emerging IVM resistance, especially in *H. contortus,* in Swedish sheep flocks. This is possibly due in part to the introduction of resistant parasites with animal imports from Europe, with resistance to IVM reported in a more recent case study on a limited number of farms but focusing on a farm on Gotland in the Baltic Sea that had imported sheep from the Netherlands [19]. Ongoing studies show that the situation has worsened recently, with more cases on mainland farms as well. On some farms, there are now strains that are multi-resistant to both BZ (albendazole) and IVM. There has also recently been a single case of triple-resistance including MOP, which appeared after just a few years of usage. Thus, on some farms the situation is alarming since the only available drug still showing efficacy is levamisole. Unfortunately there is a lack of information about the situation in goats [42].

A study in Norway based on FECRT showed that in randomly selected flocks of sheep, AR to BZ is presently at a low level (11%). However, when restricting the area to Rogaland County, eight out of ten (80%) sheep flocks, selected based on frequent deworming, application of the dose and move strategy or intensive use of grazing pasture, showed BZ resistance [43]. Recently, a case of AR to IVM has been detected in a flock of sheep in Norway [44]. AR to BZ has been detected in just one herd of goats, but only a limited number of animals have so far been tested [43].

Different surveys were conducted during 2013–2014 on Lithuanian sheep farms to detect AR [45, 46]. Based on FECRT, BZ resistance was found on three out of 15 farms (20%). Resistance to IVM was present on two out of 16 farms (13%) [45]. Based on in vitro studies, resistance to BZ was detected using two different methods. BZ-resistant GIN were found on all 25 farms investigated (100%) using EHT, and on 12 out of 17 farms (70.6%) using MALDT. AR to IVM using MALDT was detected on 13 out of 21 sheep farms (61.9%). AR to LEV using MALDT was detected on two out of six sheep farms (33%) [45, 46]. A survey to detect AR on goat farms in Lithuania was conducted in 2013. Using in vitro EHT, resistance to BZ (LD 50 > 0.1 µg/ml and LD 99 > 0.3 µg/ml) was detected on five out of seven goat farms (71%), AR to IVM was detected on all nine farms (100%), and AR to LEV was detected on two out of six farms (25%) [47].

No published studies on AR were found for other countries in the region.

Anthelmintics and treatments in Nordic-Baltic region

Several questionnaire surveys to obtain information on small ruminant management and worm control practices in northern Europe have been undertaken.

One of the first surveys of sheep farmers was conducted in Denmark in 1993. Based on a questionnaire survey, the majority of farmers (97%) used anthelmintics. The mean number of treatments per year for lambs and adult sheep was 1.9 and 2.3 respectively. BZ was the most-used anthelmintic and 81% of farmers had been using the same class of anthelmintic for three or more years [37]. IVM had only been launched on the Danish market a few years earlier. Another publication in 2014 on Danish goat herds indicated that the mean number of treatments for both kids and adult goats was around one treatment per year. In 26% of the herds, kids and adults were treated with anthelmintics ≥ 2 times per year. Anthelmintic drugs were used in 89% of the herds, and 75% of these had a predetermined treatment plan, 63% used IVM as their last treatment, 17% used BZ, 8% used other drugs and 13% did not know which drug they had used [38].

A study in Norway from 2007 revealed that visual appraisal of individual weight was the most common means of estimating the anthelmintic dose used in flocks of sheep (79%) and herds of goats (85%). However, a recent study indicated that this method for estimating the actual dose in flocks of sheep has now reduced to 28% [48]. BZ was the predominant anthelmintic class used in sheep flocks (65% in 2007), whereas BZ and ML were used equally common in dairy goat herds. In the period 2005–2007, farmers of 46% of the sheep flocks never changed the anthelmintic class. The dose and move strategy was practised in 33% of the sheep flocks [49].

An online survey was conducted on 20 conventional and 19 organic commercial sheep farms in Sweden with more than 70 ewes. Only minor differences were found between organic and conventional producers. The grazing management routines were similar, with the majority (59%) having a combination of natural pastures and arable land grazed by animal groups moved between paddocks at least three times per season (67%). The parasite control methods were also similar, with the majority (97%) relying on anthelmintics. Both ewes and lambs were usually treated one to two times per year, often at different times of the year. The most-used anthelmintic was IVM, followed by BZ (albendazole). Treatment decisions were generally based on faecal egg counts, but some farmers relied on blanket treatments. Several farms (64%) reported alternating between different drug classes at least once a year or at every deworming event. Most farms (59%) had tested whether the anthelmintics used had the expected efficacy on at least one occasion [50].

In 2015–2016, 71 sheep farmers and 37 goat farmers were surveyed in Lithuania. Based on the results from the questionnaires, chemotherapy and/or chemoprophylaxis have been used by 72% of sheep farmers and 54% of goat farmers. In Lithuania, the most-used classes of anthelmintics were ML (only IVM was used) (67% and 65%) and BZ (28% and 20%) for sheep and goat treatments respectively. LEV was used very sporadically (4% and 15%) on sheep and goat farms respectively. Sheep were usually treated twice (63%) in spring before turnout and in autumn before housing, and goats were usually treated once (41%) after the start of the housing period between November and December. Yearlings and adults were usually treated together [47, 51]. No data are available for other countries in the region.

Multidrug resistance and anthelmintic-resistant parasite genera

The evolution of multidrug resistance may lead to total anthelmintic failure [52]. Multidrug resistance is of growing concern in Nordic countries. According to data regularly collected by veterinarians for the Farm and Animal Health database in Sweden, the number of farms experiencing drug failure on occasion to both IVM and BZ is increasing, and in one case resistance to MOP as well [17]. Multidrug resistance has been detected in sheep farms in Lithuania. Using FECRT, multidrug resistance to BZ and ML was detected on a single farm. However, using in vitro tests, multidrug resistance to BZ and ML was detected on five out of 13 sheep farms (38.5%) [51]. In Denmark, using FECRT, multiple resistance to both IVM and BZ was found in seven goat herds (70%) [38]. Another study revealed an isolate of *T. colubriformis* to be resistant to both BZ and IVM [41]. Dual resistance to both IVM and BZ, based on FECRT, has also recently been recorded in single sheep flock in Norway [44]. There is a definite need for more studies on multidrug resistance in the region based on a randomised design.

In Norwegian goat herds, the pre-treatment infection levels of GIN were low compared with those in sheep flocks, and AR to BZ in *Teladorsagia* and *Trichostrongylus* was found in one goat herd [43]. In Lithuania, using different AR detection methods, *Teladorsagia* and *Trichostrongylus* were the dominant genera [45, 51]. In contrast, it appears that resistance is mainly associated with *Haemonchus* in Sweden, but other genera such as *Teledorsagia* and *Trichostrongylus* can also be involved according to droplet digital polymerase chain reaction data [53].

Based on a nematobiome study in Sweden, identified nematode species responded differently to IVM, BZ (albendazole) and LEV. Of practical interest is that both IVM and ABZ were unable to control *H. contortus* on several farms, whereas LEV still had reasonable efficacy. However, despite always having a high efficacy of treatment with LEV, *H. contortus* in particular survived at a low level. Similarly, *T. circumcincta* survived treatment with either IVM or BZ, but to a lesser extent than *H. contortus*, and not at all after LEV treatment [54]. In Denmark, AR was confirmed by the controlled efficacy test (CET) in *H. contortus* and *T. colubriformis* isolated from a farm with both sheep and goats [39].

Conclusions

In sheep and goats in the Nordic-Baltic region, AR is common observed to IVM, BZ and LEV, which are the most commonly used anthelmintics in these countries. However, MOX and LEV are highly effective in northern Europe compared with the rest of Europe. Little is known about closantel and the relatively new drug MOP because these drugs are not applied or not registered in some countries, for example in Lithuania. As elsewhere in the world, the livestock trade appears to be contributing to the spread of AR in the region and isolated cases of multidrug-resistant cases have also been reported. This is surprising given that the frequency of treatments here is much lower than in other countries where sheep production is economically more important.

As in many other regions, many studies are case reports and surveys often suffer from lack of random sampling of farms. Studies on farms have been based on FECRT, MALDT, EHT and molecular methods. Although the gold standard for AR detection is CET, it is not a commonly used method in northern Europe because studies are usually conducted on commercial farms. In brief, standard methods for AR detection are needed not only in northern Europe but worldwide, and more thorough nationwide monitoring should be instituted [20].

In northern Europe the prevailing nematodes in sheep and goats are the same as in the rest of Europe: *Haemonchus*, *Teledorsagia* and *Trichostrongylus.* On some farms, *Haemonchus* is dominant and clinical haemonchosis has increasingly been observed in recent decades. The reasons for this are unclear, but are probably related to this parasite's propensity to rapidly develop drug resistance and a general lack of awareness of the problem, possibly in combination with global warming and the increased livestock trade within the EU. The predicted patterns of climate change could alter the epidemiology of *Haemonchus* (and other strongyles) in northern Europe [55], and one obvious approach would be to undertake predictive mapping in order to be proactive in relation to impending changes in the climate that will affect this region. However, there are still some AR and GIN epidemiology data gaps in northern Europe and the Baltics, as for example no studies of this kind are available for Estonia, Latvia or Finland.

The key factors in slowing down the development of AR are now considered to be maintaining part of a parasite population in *refugia* (i.e. unexposed to drug, e.g. on pasture or in untreated animals), adopting novel non-chemical approaches, and improving pasture management and husbandry practices, and these should be included in any potential prophylactic control regimes suggested for GIN [21, 56]. However, there is little information about what level of *refugia* is required to avoid selection for resistance [57].

Improved diagnostics in wildlife ruminants, alpacas and imported small ruminants will help control the spread of AR in this region. Wild ruminants act as reservoirs for some GIN species (*Haemonchus*, *Cooperia*, *Oesophagostomum* and *Trichostrongylus*) that carry AR mutations as well as susceptible genotypes, and are a potential risk factor for transmission of AR [58–60]. This is certainly something that should be investigated in Nordic-Baltic conditions with the region's large populations of cervids and their transboundary migrations. In addition, domestic interactions through contacts with "exotic" livestock, such as alpacas, may also be a contributing factor. It has been documented in some countries that imported camelids are sometimes infected with *Haemonchus* that are refractory to anthelmintic treatments [61, 62].

In conclusion, the present situation in regards to AR in the Nordic-Baltic region is not much different from what is observed in other European countries that have more intensive sheep and goat farming. However, a collaborative effort is required in the Nordic-Baltic region to exchange information on improved diagnostics, undertake mapping and draw up guidelines for the appropriate use of new drugs (monepantel, derquantel) or drugs that are being re-introduced, such as levamisol and closantel.

Abbreviations
AR: Anthelmintic resistance; BZ: Benzimidazoles; CET: Controlled efficacy test; EC: Effective concentration; EHT: Egg hatch test; FECRT: Faecal egg count reduction test; GIN: Gastrointestinal nematodes; IVM: Ivermectin; LD: Lethal dose; LEV: Levamisole; MALDT: Micro-agar larval development test; ML: Macrocyclic lactones; MOP: Monepantel; MOX: Moxidectin.

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Authors' contributions
AB and TK developed the idea for the review, preformed the literature review, and drafted the review. IS added information about AR on goat farms in Lithuania. JH, SMT and SS added information about AR in Sweden, Denmark and Norway, respectively. SP participated in planning the review and coordinated the writing process. AB finalised the review. All the authors participated in the subsequent discussions and revisions of the entire text. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study did not require official or institutional ethical approval. The animals were handled according to high ethical standards and national legislation.

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Not applicable.

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

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Prevalence of anthelmintic resistance of gastrointestinal nematodes in Lithuanian sheep farms

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Summary

The rapid emergence of anthelmintic resistance (AR) among gastrointestinal nematodes (GIN) in Europe, particularly to benzimidazoles (BZ), levamisole (LEV), and macrocyclic lactones (ML), presents a growing challenge to sheep farming. This study aimed to assess the prevalence of resistance to BZ, IVM, and LEV in Lithuanian sheep farms. *In vitro* micro-agar larval development tests (MALDT), recognized as the most efficient *in vitro* method for large-scale epidemiological studies, were used. The research was conducted from 2021 to 2022 across 38 sheep farms throughout Lithuania. Findings indicate widespread resistance to IVM (47.4 %) and BZ (39.5 %), while LEV resistance was not detected. Multidrug resistance (MDR) to both IVM and BZ was found in 28.9 % of farms. *Trichostrongylus*, *Teladorsagia*, and *Haemonchus* were identified as resistant species. Our results reveal a lower prevalence of AR compared to previous studies, highlighting the critical importance of randomized sampling. The study underscores the need for strategic anthelmintic management and enhanced cooperation among researchers, veterinarians, and farmers to mitigate the spread of AR in Lithuanian sheep farms.

Keywords: anthelmintic resistance; gastrointestinal nematodes; benzimidazoles; ivermectin; levamisole

Introduction

The global emergence of nematodes resistant to benzimidazoles (BZ), levamisole (LEV), ivermectin (IVM), and moxidectin (MOX) is expanding rapidly, particularly in Europe, though there remain knowledge gaps in certain regions (Beleckė *et al*., 2021; Papadopoulos *et al*., 2012; Vineer *et al*., 2020). Several factors contribute to the development of anthelmintic resistance (AR), including the high frequency of anthelmintic treatments, underdosing of anthelmintics, and lack of rotation among anthelmintic groups (Sargison *et al*., 2007). Infections with gastrointestinal nematodes (GIN) can lead to symptoms ranging from diarrhea and digestive issues to anemia and, in severe cases, animal death. However, most infections are subclinical, resulting in decreased productivity (Beleckė *et al*., 2021). Subclinical infections lead to reduced growth and lower milk and wool production, causing significant economic impact. Moreover, parasitic worms affect animal welfare and, indirectly, human well-being through impacts on farm management, production, and food security (Charlier *et al*., 2023). The total cost of helminthic infections in sheep farming is estimated at €357 million, with

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resistance to MOX alone costing approximately €38 million annually in Europe, and this cost is increasing. Since 2010, the average prevalence of AR in sheep and goats in Europe has been 86 % for BZ, 52 % for macrocyclic lactones (ML), 48 % for LEV, and 21 % for MOX (Charlier, J. *et al*., 2020; Vineer *et al*., 2020).

The presence of nematode strains resistant to BZ, IVM and ML has been repeatedly reported, particularly for the three genera: *Haemonchus*, *Teladorsagia* and *Trichostrongylus* (Papadopoulos *et al*., 2012). However, multidrug resistance (MDR) has been identified in 10 Europe countries, but likely to be more widespread than reported (Charlier, J. *et al*., 2022; Vineer *et al*., 2020).

Various *in vitro* tests are available to detect AR with the Egg Hatch Test (EHT) being the most commonly used. However, the Larval Development Test (LDT) is considered the most efficient for detecting resistance to BZ, LEV, and IVM, despite being more laborious and time-consuming. LDT is applicable for detecting resistance to all broad-spectrum anthelmintics, including macrocyclic lactones, whereas EHT is limited to BZ resistance detection. *In vitro* tests have shown higher sensitivity, identifying even as low proportions of resistant parasites within a population as 4 % (Babják *et al*., 2021; Várady *et al*., 2007).

The *in vivo* fecal egg count reduction test (FECRT), recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP), is effective but costly and time-consuming, requiring multiple tests on herd animals, which often makes it impractical for farmers (Kaplan *et al*., 2023; Mickiewicz *et al*., 2021). Assessing all five licensed anthelmintic groups, including IVM and MOX, along with a control group, demands a substantial number of animals, further limiting feasibility for commercial farms. As a result, most studies evaluate only a limited range of anthelmintics. While the controlled *in vivo* efficacy test is the gold standard for anthelmintic resistance assessment, it's reliance on animal slaughter makes it unsuitable for commercial farm monitoring (Vineer *et al*., 2020). Additionally, molecular tests remain limited to detecting BZ resistance (Coles *et al*., 2006).

AR research often lacks studies with large, randomly selected samples, typically relying on farmer self-selection or samples from regions near universities due to resource limitations. The median sample size for AR studies is five farms (Vineer *et al*., 2020). In most Nordic-Baltic countries randomized studies have not been carried out (Beleckė *et al*., 2021). In Norway, lower BZ resistance was found in randomly selected farms compared to non-randomly selected ones (Domke *et al*., 2012). Only a few studies using *in vitro* and *in vivo* tests have been carried out in central and southern parts of Lithuania. They involved non-randomly selected farms usually with a history of regular use of anthelmintics (Kupčinskas *et al*., 2016; Kupčinskas *et al*., 2015a; Kupčinskas *et al*., 2015b).

This nationwide randomized study aimed to investigate the prevalence and current status of resistance of GIN to BZ, IVM, and LEV in Lithuania using the *in vitro* micro-agar larval development test (MALDT).

Materials and Methods

Study design

The study was conducted in years 2021 – 2022 and sheep farms were randomly selected to participate in the survey. The only inclusion criterion was that farms must have at least 15 sheep to ensure a representative sample of actively managed flocks with established anthelmintic treatment practices. A total of 38 sheep farms, representing 19.7 % of the 193 farms invited by the Lithuanian Sheep Breeders Association, agreed to participate. The sample size was determined based on practical feasibility and insights from similar studies (e.g., Dolinska *et al*., 2014; Kupčinskas *et al*., 2015b). A convenience-based sampling approach was employed to ensure the inclusion of a sufficient number of farms for meaningful statistical comparisons.

Farmers were asked to complete a structured questionnaire providing information on farm size, sheep breeds, the purchase of sheep from other farms or abroad, and deworming history over the past three years, including the frequency and types of drugs used.

To assess AR, the in vitro MALDT to detect resistance to BZ, IVM, and LEV. These anthelmintics were selected based on previous surveys indicating them as the most commonly used in Lithuania (Kupčinskas *et al*., 2016).

On each farm, faecal samples were collected directly from the rectum of 15 – 20 randomly selected sheep. Pooled faecal samples weighing 50 – 100 g were stored anaerobically in plastic tubes filled with water at room temperature and processed within two days.

Nematode eggs were extracted by sieving the feces through a series of sieves with decreasing aperture sizes (250, 100, and 20 μm). The material retained on the 20-μm sieve was washed with tap water, sedimented, and further processed using the salt flotation method to recover trichostrongylid eggs for the *in vitro* MALDT (Dolinska *et al*., 2014).

In vitro test

The MALDT, as described by Coles *et al*. (2006), was used. Tests were conducted in 96-well microtiter plates. Stock solutions of ivermectin aglycone (IVM-Ag) and thiabendazole (TBZ) were serially diluted 1:2 with dimethyl sulfoxide (DMSO) and LEV was diluted with deionized water to produce 12 concentrations ranging from 0.084 to 173.6 ng/ml for IVM-Ag, 0.0006 to 1.28 μg/ml for TBZ and from 0.0156 to 32 μg/ml for LEV. Then, 12 μl of each stock solution at different final concentrations were mixed with 150 μl of 2 % Bacto agar (Difco, USA) and stored at 4 °C for 5 min. To inhibit fungal growth, 10 μl of eggs (final number of 50 eggs per well) in a 0.3 mg/ml solution of amphotericin B (Sigma-Aldrich, Germany) were mixed with 10 μl of yeast extract and added to the agar (Kupčinskas *et al*., 2015a; Kupčinskas *et al*., 2015b). The yeast extract was prepared as described by Hubert and Kerboeuf (Hubert & Kerboeuf, 1984) (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). DMSO

(1.3 %) was used in the control wells. The plates were incubated for 7 days at 27 °C. Incubation was terminated by adding Lugol's iodine solution to each well. After incubation, the proportions of unhatched eggs, L, L2 and L3 stage larvae at each concentration were determined under an inverted stereomicroscope (Kupčinskas *et al*., 2015a; Kupčinskas *et al*., 2015b). The L3 larvae in the discriminating concentration were differentiated and identified by morphological features (Van Wyk *et al*., 2004).

Statistical analysis

Data were analyzed using a threshold discriminating concentration instead of conventional threshold values (LC50 or LC99). Farms were classified as resistant when L3 larvae were found at concentration 21.6 ng/ml for IVM-Ag, 0.04 µg/ml for TBZ and 2 µg/ml for LEV (Babják *et al*., 2018; Dolinska *et al*., 2014; Kupčinskas *et al*., 2015a; Kupčinskas *et al*., 2015b; Taylor, 1990). The percentage of developed L3 larvae at the threshold concentration was categorized as mild (< 10 %), moderate (10 – 50 %), and severe (> 50 %) (Mickiewicz *et al*., 2021).

Statistical analyses were performed using "SPSS for Windows version 20". Descriptive statistics were calculated, and comparisons between groups were made using the Mann-Whitney U test, Pearson's chi-square, or Fisher exact test. The significance level was set as 0.05. The 95 % confidence intervals (CI 95 %) were calculated using one-sample binomial success rate (Clopper-Pearson).

Results

Sheep farms characteristics

The study included 38 farms located throughout Lithuania, covering 8 out of 10 provinces. The size of the farms varied from 15 to 1500 adult sheep, with a median of 84 sheep. Most farms kept non-Lithuanian sheep breeds (47.4 %), including Romanov, Suffolk, German Black-headed, Sarole, Merinofleischschaf, Berichon du Cher, and Texel. Lithuanian local breeds, such as Lithuanian Black-headed sheep and Lithuanian Coarse, were maintained on 36.8 % of the farms, while the remaining 15.8 % kept crossbreeds. Three farms had imported sheep from abroad in the past three years. The majority of farms dewormed their sheep twice a year (n=23; 60.5 %; CI 95 %: 43.4 %, 76.0 %), while 8 farms (21.1 %; CI 95 %: 9.6 %, 37.3 %) dewormed once a year and 3 farms (7.9 %; CI 95 %: 1.7 %, 21.4 %) dewormed three times a year. Four farms (10.5 %; CI 95 %: 2.9 %, 24.8 %) did not engage in routine deworming. The most commonly used anthelmintic was IVM used by 22 farms (57.9 %; CI 95 %: 40.8 %, 73.7 %). Ten farms rotated IVM with BZ (26.3 %; CI 95 %: 13.4 %, 43.1 %), and one farm rotated IVM with LEV and BZ (2.6 %; CI 95 %: 0.07 %, 13.8 %). Only one farm (2.6 %; CI 95 %: 0.1 %, 13.8 %) used LEV exclusively. Additionally, only 8 farmers (21.1 %; CI 95 %: 9.6 %, 37.3 %) regularly consulted with veterinarians and conducted deworming tests.

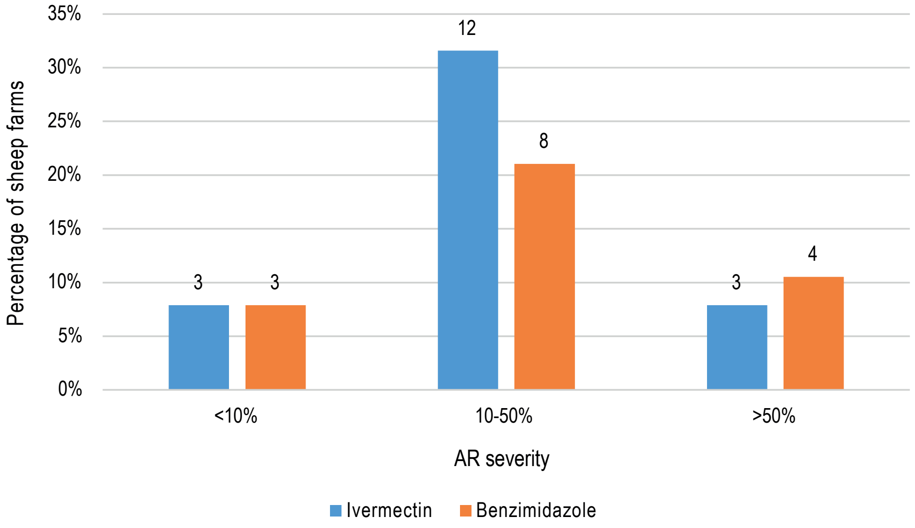

Fig. 1. Number of sheep farms where the development of L3 larvae was present at the threshold concentration 21.6 ng/ml for IVM-Ag and 0.04 µg/ml for TBZ in sheep farms in Lithuania

3

Micro-agar larval development test

The results MALDT are presented in Figure 1. The most prevalent resistance was to IVM. L3 larvae development at the IVM threshold concentration indicated resistance in 18/38 farms (47.4 %; CI 95 %: 31.0 %, 64.2 %). Among these, moderate levels of IVM resistance were detected in 12 farms (66.7 %), while 3 farms exhibited mild (16.7 %) and another three severe (16.7 %) levels of AR. BZ resistance was less common, detected in 15/38 farms (39.5 %; CI 95 %: 24.0 %, 56.6 %). Among these, 8 farms (53.3 %) had moderate levels of BZ resistance, 4 farms (26.7 %) had severe levels of BZ resistance, and 3 farms (20.0 %) had mild levels of BZ resistance. LEV resistance was not detected in any of the Lithuanian sheep farms (0 %; CI 95 %: 0 %, 9.3 %). Notably, eight farms on which BZ had not been used in previous three years showed BZ resistance. Similarly, one farm showed IVM resistance despite no reported use.

Sixteen farms (42.1 %) were free from resistance to all anthelmintics. Resistance to at least one anthelmintic was detected on 22/38 farms (57.9 %; CI 95 %: 40.8 %, 73.7 %) - resistance to a single anthelmintic on 11/38 farms (28.9 %; CI 95 %: 15.4 %, 45.9 %) and AR to BZ and IVM on another 11/38 farms (28.9 %; CI 95 %: 15.4 %, 45.9 %). MDR was significantly associated with non-Lithuanian sheep farms breeds (p = 0.007).

BZ resistance was significantly associated with farms raising either Lithuanian local breeds or non-Lithuanian breeds (p = 0.001), but this was not the case for IVM resistance. However, no significant relationship was found between crossbreeds and the use of BZ or IVM. The use of BZ was significantly linked to BZ resistance, whereas IVM use was not significantly related to IVM resistance. The frequency of deworming per year did not link with the intensity of AR for any anthelmintic (IVM: p = 0.103, BZ: p = 0.234).

L3 larvae were differentiated at the discriminating concentration. In the case of BZ resistance, *Trichostrongylus spp.* was found on 8/15 farms (53.3 %; CI 95 %: 26.6 %, 78.7 %), in 6 farms (40.0 %; CI 95 %: 16.3 %, 67.7 %) – *Teladorsagia circumcincta*, and in 1 farm (6.7 %; CI 95 %: 0.2 %, 31.9 %) – *H. contortus*. In the case of IVM resistance, *Trichostrongylus spp.* was identified on 10/18 farms (55.6 %; CI 95 %: 30.8 %, 78.5 %), in 2 farms (11.1 %; CI 95 %: 1.4 %, 34.7 %) *T. circumcincta*, and in 2 farms (11.1 %; CI 95 %: 1.4 %, 34.7 %) – *H. contortus*. In one farm where *H. contortus* was found, MDR was present. In other MDR cases presence of *Trichostrongylus* (5/11; 45.5 %; CI 95 %: 16.7 %, 76.6 %) and *Teladorsagia* (5/11; 45.5 %) was revealed.

Discussion

The prevalence of AR has shown an upward trend over the past 41 years, with a gradual increase for BZ and a more abrupt rise for ML (Vineer *et al*., 2020). This study is the first nationwide randomized survey in Lithuania, without any criteria related to previous anthelmintic treatment. According to Vineer at al. (2020), non-random sampling, such as researchers selecting farms with suspected AR or farmers self-selecting due to suspicion of AR on their farms, can inflate prevalence estimates. Often, studies sample single farms near universities or research institutes, with a median sample size of only five farms for AR studies on GIN (Domke *et al*., 2012). In northern Europe, many studies are case reports and surveys often lack random sampling of farms (Belecké *et al*., 2021).

The first study in Lithuania to investigate IVM resistance using the MALDT was conducted in 2013 – 2014, documenting IVM resistance in 13 out of 21 farms (61.9 %). BZ resistance, detected using the EHT, was found in all farms studied (Kupčinskas *et al*., 2015a). A subsequent study in 2014 using MALDT found BZ resistance in 12 out of 17 farms (70.6 %) and LEV resistance in 2 out of 6 farms (33.4 %) (Kupčinskas *et al*., 2015b). These studies were limited to central and southern Lithuania and focused on farms with a history of anthelmintic use (Kupčinskas *et al*., 2015a; Kupčinskas *et al*., 2015b). Our current results indicate a lower presence of AR to BZ and IVM in Lithuania compared to previous studies, with no detected resistance to LEV. This aligns with findings from northern Europe, where moxidectin and LEV remain highly effective compared to the rest of Europe (Belecké *et al*., 2021). Moreover, levels of AR on GIN to BZ and IVM become lower than in previous studies. This could lead to a wider random study. Only one randomly selected study in Europe was performed and the result showed that randomly selected farms resistance of BZ was lower than in non-randomly selected farms (Domke *et al*., 2012).

Interestingly, eight farms reported not using BZ in the last three years, yet BZ resistance was detected in these farms. A similar situation was observed on one farm with IVM resistance. The same situation was described in Polish goat herds where 3 herds were resistant to LEV, 9 to ML, and 3 to BZ (Mickiewicz *et al*., 2021). As explained by Mickiewicz *et al*. (2021) this discrepancy could arise from farmers' unawareness or forgetfulness regarding anthelmintic use or from the acquisition of animals previously treated with these anthelmintics. Lithuanian farmers were also not fully aware of the anthelmintics they used. 10.5 % did not use routine deworming or they had forgotten the fact of using the drugs in their farms. Given the small size of Lithuania and the common practice of transitioning sheep between farms, the introduction of resistant GIN populations from other farms with unknown deworming histories is plausible. As well as there is also known cross-resistance in which a parasite strain can tolerate the therapeutic doses of anthelmintics that are unrelated chemically or anthelmintics having different mechanisms of action (Fissiha & Kinde, 2021). The mechanisms of resistance to BZ, LV, and ML are different, and there is virtually no cross-resistance between them (Bartram *et al*., 2012; Coles & Roush, 1992; de Lourdes Mottier & Prichard, 2008). However, Mottier and Prichard (2008), described the study on *H. contortus* which showed correlation between exposure and/or resistance to MLs and an increase in the frequency of the b-tubulin alleles containing codons, which are determinants for benzimidazole resistance. This means that ML use may predispose parasitic nematodes to benzimidazole resistance.

The prevalence of MDR in Lithuania remains a significant concern. In a previous study, MDR to BZ and ML was detected in five out of 13 sheep farms (38.5 %) using the FECRT (Kupčinskas *et al*., 2016). Our study, also employing FECRT, found MDR to IVM and BZ on 11 out of 38 farms (28.9 %). While the percentage of farms with MDR appears lower in the current study, this may be explained by differences in study size and the larger number of farms investigated. Furthermore, MDR remains a persistent issue, consistent with trends observed in other countries. MDR to common anthelmintics has been reported in Scotland (Sargison *et al*., 2007), the United Kingdom (Taylor *et al*., 2009), Ireland (Keegan *et al*., 2015), Denmark (Holm *et al*., 2014), Norway (Odden *et al*., 2018), France (Paraud *et al*., 2009), Greece, Italy (Geurden *et al*., 2014), and Austria (Untersweg *et al*., 2021). These findings highlight the emerging and ongoing challenges posed by MDR in gastrointestinal nematodes worldwide.

In Lithuania, the most prevalent resistant GIN genera are *Trichostrongylus, Teladorsagia*, *Haemonchus*. Previous study in Lithuania revealed the presence of *Teladorsagia* and *Trichostrongylus* spp. in all MALDT tests (Kupčinskas *et al*., 2015b). European studies show that the most common resistant species are *Haemonchus*, *Teladorsagia*, *Trichostrongylus*, *Cooperia,* and *Nematodirus* (Charlier *et al*., 2022; Kenyon *et al*., 2009; Vineer *et al*., 2020).

Only 38 out of 193 invited farms (19.7 %) participated in the study, reflecting a low response rate that may be influenced by several factors specific to Lithuania. Time constraints could play a significant role, as farmers often face demanding daily routines, particularly during peak agricultural seasons, leaving them with limited availability for participation in studies. Distrust in institutions is another important factor, with some farmers expressing skepticism toward governmental institutions, researchers, or scientific studies. Concerns about data confidentiality and how the information will be used may further discourage participation. Additionally, a lack of interest in scientific research is observed in certain farming communities, where there is a tendency to prioritize initiatives that provide immediate financial or practical benefits. A unique contextual factor in this study was farmers' focus on other priorities. During the data collection period, many farmers were more engaged in advocating for the reduction of wolf populations, which they considered a more urgent issue than contributing to scientific research.

Conclusions

This study demonstrates that AR of GIN to BZ and IVM is widespread in Lithuanian sheep farms, whereas LEV remains highly effective. The prevalence of AR is not as high as previously reported, underscoring the importance of randomized studies. To prevent the development of AR in Lithuanian sheep farms, an appropriate strategy for anthelmintic treatment is crucial. Collaboration among researchers, veterinarians, and farmers is essential to halt the spread and reduce the levels of AR.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Gastrointestinal Nematode Control in Lithuanian Sheep Farms: Insights from a Questionnaire Survey

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Simple Summary: Gastrointestinal parasite infections are a major challenge in sheep farming, affecting animal health, welfare, and farm productivity. Anthelmintic drugs, commonly used to control these parasites, can become less effective when used too frequently or without proper diagnostic guidance, leading to the development of resistance. This study assessed parasite control practices on Lithuanian sheep farms and explored factors that may promote resistance. A survey of 71 farmers showed that most used anthelmintics routinely, mainly twice per year, but only a small proportion based treatment decisions on diagnostic testing. Organic farms were more likely to use diagnostic methods than traditional farms. Many farmers relied on the visual estimation of sheep weight rather than accurate weighing when determining dosages, increasing the risk of underdosing. Macrocyclic lactones were the most commonly used type of anthelmintic. Quarantine measures for newly introduced animals were not consistently applied. These findings highlight a heavy reliance on routine, non-strategic treatments, and limited use of evidence-based approaches. Strengthening farmer education on parasite management, promoting regular diagnostic testing, accurate dosing, and effective quarantine procedures are essential steps to slow the development of anthelmintic resistance and to support sustainable sheep farming practices.

Abstract: Anthelmintic resistance (AR) in gastrointestinal nematodes is a growing global concern that threatens effective parasite control in livestock farming. This study aimed to assess gastrointestinal nematode (GIN) control practices and identify risk factors associated with AR development on Lithuanian sheep farms. A cross-sectional survey was conducted between 2022 and 2023 among 71 members of the Lithuanian Sheep Breeders Association, covering farm management, grazing, and deworming practices. Anthelmintics were used by 90.2% of farmers, with most treating their flocks twice a year. Only 18.3% of farmers performed parasitological testing to guide treatments, with significantly more organic farms applying this approach than traditional farms. Treatment frequencies were significantly higher in traditional farms. Most farmers (45.1%) relied on the visual appraisal of sheep weight for dosing, while 35.2% dosed according to the weight of a medium-sized animal. Macrocyclic lactones were the most commonly used anthelmintics (50.7%). Quarantine procedures for new animals were implemented by less than half of the farmers (44.4%), while others treated without isolation (56.6%). These findings indicate reliance on non-strategic parasite control methods and limited use of diagnostics, highlighting the need for improved farmer education to promote sustainable parasite control and reduce AR risk in Lithuanian sheep farms.

Keywords: anthelmintic resistance; gastrointestinal nematodes; sheep farming; survey; worm control

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1. Introduction

Gastrointestinal nematode (GIN) infections are a major issue affecting the health and productivity of small ruminants globally. These parasites cause significant economic losses due to reduced growth rates, decreased wool production, and impaired reproductive efficiency [1–3]. In response, the widespread use of anthelmintics has been the primary strategy for nematode control. However, over-reliance on these drugs has led to the emergence of anthelmintic resistance (AR), which poses a growing threat to sheep farming sustainability [1,4]. Nematode resistance to benzimidazoles (BZ), levamisole (LEV), ivermectin (IVM), and other macrocyclic lactones (ML) is becoming increasingly widespread globally, with resistance expanding in many parts of Europe. However, knowledge gaps remain in certain regions, particularly in Eastern Europe [3,5]. This region has a limited number of registered anthelmintics [3]. The average prevalence of resistance at the farm level was found to be 86% for BZ, 52% for ML excluding moxidectin (MOX), 48% for LEV, and 21% for MOX [5]. Multiple cases of AR in GIN of sheep have been identified in France, Greece, and Italy [6]. Additionally, multidrug resistance (MDR) has been documented in the UK and Ireland, and it is becoming an increasing Scotland [7–9] and France [10].

Questionnaire-based surveys on worm control practices in small ruminants have previously been conducted in Denmark [11–13], the United Kingdom [14–16], France [17], Slovakia [18], Norway [19,20], Sweden [21,22], and Lithuania [23]. Helminth control strategies must now include management practices that focus on extending the effectiveness of anthelmintics. This is especially crucial given the low likelihood of new active compounds being introduced in the near future, and the fact that commercially available subunit antihelminth vaccines are still a long way off [24]. It is now evident that in order to ensure long-term profitability in livestock production, sustainable parasite control programs must incorporate integrated novel non-chemical methods, enhanced pasture management and husbandry practices, along with strategies that manage refugia [1]. For the best practices to be effectively implemented, it is essential that farmers receive proper guidance on parasite management strategies, with a focus on the strategic use of anthelmintics. Implementing integrated parasite control strategies that rely less on chemical anthelmintics is complex due to various factors, including differences in local parasite species, host breeds, climate, management practices, and traditions. Additionally, regional and farm-specific drug resistance further complicates the situation. The lack of data on the best use of anthelmintics to balance animal health, productivity, and resistance prevention adds to these challenges [25].

There is currently a lack of comprehensive data on worm control practices and livestock management on sheep farms in Lithuania. Gaining knowledge in this area is essential for developing effective guidelines to prevent anthelmintic resistance. Therefore, the objective of this study was to expand our knowledge of worm control practices on sheep farms in Lithuania.

2. Materials and Methods

This study utilized a cross-sectional questionnaire survey to collect data on the farming practices of members of the Lithuanian Sheep Breeders Association. The survey was conducted over a two-year period from 2022 to 2023. The participants in this study were all members (193) of the Lithuanian Sheep Breeders Association, which encompasses the entire territory of Lithuania. Data collection was performed via telephone interviews with each member. The interviews were conducted by the authors themselves. Of the 193 members contacted, 71 participants completed the questionnaire (response rate: 36.8%). Among the remaining individuals, 73 did not answer the phone despite repeated call attempts, and 49 explicitly refused to participate in the survey. Verbal informed consent was obtained prior to each interview, followed by the mailing of a written consent form to all participants for documentation purposes.

The questionnaire consisted of 16 questions (Supplementary Materials) and was developed specifically for this study. It included 9 multiple-choice and 7 open-ended items, designed to collect detailed information on key aspects related to parasite control in sheep farms. The questionnaire covered the following thematic areas: (1) Farm characteristics (4 questions); (2) Grazing practices (3 questions), focusing on the duration and system of grazing; and (3) Worm-control practices (9 questions), addressing the timing and frequency of treatments, types and dosages of anthelmintic products used, and quarantine measures applied for newly introduced animals.

Data were analyzed using "SPSS for Windows version 20". Descriptive statistics were employed to summarize the data. To provide a measure of precision around proportions, 95% confidence intervals (CI) were calculated for categorical variables. To assess statistical significance, Chi-square tests were used to compare proportions between categorical variables. When expected cell frequencies were less than five, Fisher's Exact Test was applied to ensure statistical validity. Comparisons of continuous variables, including average treatment frequency, between traditional and organic farms were performed using independent samples *t*-tests. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. General Information

During 2022–2023, all 193 members of the Lithuanian Sheep Breeders Association, geographically distributed across all 10 counties of Lithuania, were surveyed, with the highest proportion of respondents from Utena County (13/71, 18.31%), followed Kaunas and Marijampolė Couties (11/71, 15.49% each) (Figure 1). The questionnaire was completed by 71 members, resulting in a response rate of 36.8% (95% CI: 29.9–44.1%).

Figure 1. Regional distribution of surveyed sheep farms across Lithuania's 10 counties.

Among the respondents, 60.6% of farms were organic and 39.4% were traditional (95% CI: 48.8–71.5% and 28.5–51.2%, respectively). The average flock size across these farms was approximately 184.5 sheep (range: 10–1600). Rotational grazing was practiced on 36.6% (*n* = 26, 95% CI: 25.6–48.7%) of the farms surveyed, while 63.4% (*n* = 45, 95% CI: 51.3–74.4%) kept sheep on a single pasture throughout the grazing period. The predominant sheep breed was Lithuanian Black Head, present in 46.5% (*n* = 33, 95% CI: 34.9–58.3%) of farms, followed by mixed breeds (18.3%), Merinolandschaf (5.6%) and Suffolk (5.6%). Grazing periods began primarily in May (*n* = 34), followed by April (*n* = 30) and March (*n* = 7), and ended mostly in November (*n* = 54), with fewer in December (*n* = 16) and October

(n = 1). Most farms (87.3%, 95% CI: 77.9–93.9%) did not keep other animals. Among those that did, 8.5% kept goats, 2.8% cattle, and 1.4% horses. The average grazing area was 34.51 hectares, ranging from 1 to 200 hectares. Water supply methods varied: 67.6% (n = 48, 95% CI: 55.3–78.2%) of farms used well water, while the rest relied on access to natural moist areas in pastures combined with other on-farm water sources.

3.2. GIN Treatment Strategies

Only 18.3% (n = 13, 95% CI: 10.1–29.1%) of farmers used parasitological analysis of fecal samples as an indicator for treatment effectiveness. Organic farms were more likely to utilize this method, with 23.3% (n = 10, 95% CI: 12.4–37.2%) conducting parasitological tests, compared to only 10.7% (n = 3, 95% CI: 2.3–28.2%) of traditional farms ($p < 0.05$). The main factors influencing decisions on when to treat against helminths were previous experience (38.0%) and the occurrence of diarrhea (22.5%). The spring before turn-out and autumn before housing were the most common times for sheep treatment. It was estimated that 90.2% of sheep farmers (n = 64, 95% CI: 81.4–95.7%) used anthelmintics to control gastrointestinal nematodes. Based on the questionnaire results, lambs were drenched on average 1.54 times per year, and yearlings/ewes 1.49 times per year. Annual treatment frequencies showed that most farms (49.3%) drenched their sheep twice per year, 38.0% treated once per year, and 4.2% treated three times per year. Additionally, 8.5% of farmers reported that they do not treat their sheep at all. A few farmers treated lambs (8.5%) and yearlings/ewes (4.2%) more than twice a year (Table 1). No statistically significant differences were observed between farm types in annual treatment frequency ($p > 0.05$).

Table 1. Frequency and distribution of anthelmintic treatments in sheep flocks by age group (n = 71).

	Number of Treatments	%	95% CI
Yearlings/ewes	1	38.0%	27.3–49.4%
	2	49.3%	37.9–60.7%
	3	4.2%	0.9–11.7%
	Never	8.5%	3.2–17.5%
Lambs	1	3.2–17.5%	22.9–44.3%
	2	37.8%	27.2–49.4%
	3	8.5%	3.5–16.7%
	Never	7.3%	2.8–15.1%

In comparing treatment frequencies between traditional and organic farms, notable differences were observed. The average drenching rate for yearlings/ewes in traditional farms was 1.71 treatments per year, which is approximately 1.27 times higher than the 1.35 treatments per year observed in organic farms. This difference was statistically significant ($p < 0.05$). Similarly, the treatment rate for lambs in traditional farms was 1.71 treatments per year, approximately 1.19 times higher than the 1.44 treatments per year in organic farms, which was also statistically significant ($p < 0.05$). For anthelmintic dose estimation, most farmers (45.1%) relied solely on the visual appraisal of sheep weight. Meanwhile, 35.2% weighed a medium-sized animal and dosed the whole flock based on that weight (Table 2).

ML were the most frequently used anthelmintic class, reported by 50.7% of farms. BZ were used by 11.3% of farms (Table 3). Rotation between BZ and ML was practiced in 16.9% of farms. Furthermore, 12.3% of farmers reported purchasing new sheep from abroad. However, less than half (44.4%) implemented quarantine measures upon arrival, while 56.6% opted only to drench newly purchased sheep without quarantine. The difference between implementing quarantine and drenching without quarantine was statistically significant ($p < 0.05$).

Table 2. Anthelmintic dose estimation methods in the sheep flocks (*n* = 71).

Calculation or Estimation of Weight	%	95% CI
Visual appraisal of weight	45.1%	33.6–56.9%
Weighing a medium sized sheep	35.2%	24.6–47.2%
Weighing the largest sheep	5.6%	1.5–13.6%
Weigh each animal	2.8%	0.3–9.7%
No treatment	11.3%	5.0–21.0%

Table 3. Use of anthelmintics against GINs over a three-year period based on farmer responses (*n* = 71), including ML, LEV, BZ, and rotation of anthelmintic classes.

Anthelmintic	%	95% CI
ML	50.7%	39.4–62.0
LEV	5.6%	1.4–11.3
BZ	11.3%	4.2–19.7
BZ + ML	16.9%	8.5–25.4
BZ + ML + LEV	7.0%	1.4–14.1
No treatment	8.5%	2.8–15.5

4. Discussion

GIN infections present a significant challenge to sheep farming globally, and Lithuania is no exception. The results of this study provide important insights into the worm control practices on Lithuanian sheep farms. The response rate of 36.8% from the Lithuanian Sheep Breeders Association members provides a substantial dataset, allowing for the characterization of current practices and the identification of potential areas for improvement. However, the relatively low response rate may be attributed to several factors, such as limited farmer engagement in scientific surveys, lack of time or interest, and possible concerns regarding the confidentiality of shared information. Additionally, some farmers may not perceive gastrointestinal nematode infections as a pressing issue, which could further reduce their motivation to participate.

The study found that 36.6% of the farms practiced rotational grazing, while a majority, 63.4% kept their sheep on a single pasture with a shelter throughout the grazing period. The minority of respondents (12.7%) practiced mixed or alternate grazing with cattle or horses, a well-known strategy for reducing parasite pressure and minimizing the reliance on anthelmintics for parasite management [26].

The findings indicate the limited use of parasitological diagnostics among sheep farmers, particularly in traditional farms, where treatment decisions were less evidence-based. Higher drenching frequencies observed in traditional farms suggest a more intensive, possibly prophylactic approach, which may increase the risk of developing anthelmintic resistance. Only 18.3% of farmers utilized parasitological analysis of fecal samples to guide treatment, while the majority relied on past experiences (38.0%) or visible symptoms like diarrhea (22.5%), similar to reports from several European countries [15,19,27]. In Belgium, only few sheep farmers (9%) regularly used fecal egg counts to monitor worm infections [28]. This dependence on subjective indicators rather than objective diagnostic methods highlights a critical area for improvement in worm control practices in Lithuania. Greater access to and use of fecal egg count monitoring could enable more targeted treatments, potentially reducing anthelmintic use and slowing the development of AR. According to a study in Norway [19], 90% of farmers did not suspect treatment inefficacy, so changing established practices might not be seen as necessary. Farmers may undervalue diagnostic testing if their treatment routines yield acceptable results, viewing it as an unnecessary expense. With multiple farming challenges like time management, livestock welfare, and regulatory compliance, parasitological testing may not be a top priority, especially if anthelmintic treatment seems effective. Additionally, a lack of encouragement from veterinarians could contribute to low testing rates.

A prior study by Kupčinskas [23] found that the majority of sheep farmers in Lithuania (92.2%) determined the correct anthelmintic dosage by visually estimating the weight of their sheep. It appears that the situation has improved in recent years, as visual appraisal is now used less frequently (45.1%). Similar results were found in Norway, Sweden, Slovakia, and France, where the visual appraisal of weight was the dominant choice of dose [17,18,20,21]. Incorrect live-weight estimation and improper calibration of drench guns can lead to under-dosing. To ensure proper dosing, it is important to estimate animal weight as accurately as possible, ideally by individually weighing each animal. Alternatively, weighing the heaviest animal and slightly overestimating the dose for all animals can also help ensure correct anthelmintic treatment [20]. In our survey, if weighing each animal or only the heaviest was considered the only acceptable method for dose calculation, only 8.5% of sheep flocks had accurate dose calculations. Burgess et al. [29] suggested that underdosing with substances from the same class might be more critical than drenching frequency, which was found to be low in our survey.

Compared to a previous study [23], the use of anthelmintics in Lithuania has significantly increased from 71.8% to 90.2%, although the drenching rates for lambs and ewes have been increased slightly, from 1.24 to 1.54 and from 1.29 to 1.49 times per year. This drenching rate is slightly lower compared to other European countries. Domke et al. reported mean drenching rates of 2.5 for lambs and 1.9 for ewes in Norway [20], while in Denmark, the rates were 1.9 for lambs and 2.3 for ewes [12]. In the Netherlands, ewes were treated on average 1.53 times per year, while lambs received 2.05 treatments annually [27]. In Belgium, both ewes and lambs were treated more frequently, with mean annual drenching rates of 2.6 and 3.2, respectively [28]. In Scotland and England, the mean drenching rates are higher, ranging from 2.2 to 4.4 treatments per lamb annually [15,30]. The average drenching frequency in France was 5.2 times per year [31], compared to 1.70–1.76 times per year in the Slovak Republic [18]. In Sweden, the use of anthelmintics was relatively low, at 45%, with the majority (76%) of those treatments involving ewes being drenched on a single occasion [21]. Frequent treatments are regarded as a significant risk factor for the development of anthelmintic resistance [32]. Moreover, farmers who treated their ewes more frequently also tended to treat their lambs more frequently [27].

A significant finding from this study is the high prevalence of organic farming (60.6%) compared to traditional systems (39.4%), highlighting the prominence of sustainable practices in Lithuania. Organic farms were more inclined to use parasitological analysis, with 23.3% conducting tests, in contrast to just 10.7% of traditional farms. Variations between traditional and organic farms suggest differing approaches to disease management. The observation that traditional farms drench more frequently than organic farms reflects inherent differences in disease management strategies. Organic farming often emphasizes natural resistance and lower intervention, which is reflected in the lower treatment rates. In contrast, traditional farms, perhaps due to more intensive farming practices, seem to rely more heavily on chemical interventions to manage parasite infection.

ML were the most commonly used anthelmintic class on Lithuanian sheep farms, with 50.7% of farmers reporting their use. This represents a slight decline from the previous study, where ML usage was 56.9% [23]. Similarly, the use of (BZ) also saw a decline, dropping from 15.7% [23] to 11.3%. In contrast, the use of (LEV) showed a modest increase, rising from 3.9% [23] to 5.6%, though it is still used sporadically. This may indicate a potential over-reliance on specific anthelmintic classes, which could accelerate the development of resistance. The choice of anthelmintic drugs in Lithuania appears to align with Northern

European countries but differs from other parts of Europe, where there is growing use of ivermectin, along with increased reliance on closantel, doramectin, moxidectin, and combination treatments [3,16,21,23]. Anthelmintic rotation was used only in 23.9% of farms and IVM, BZ, and LEV are the only single substances registered against GIN of sheep in Lithuania. The Nordic–Baltic region has a limited number of registered anthelmintics [3]. However, animal welfare requirements are stringent, and anthelmintics can only be sold with a prescription.

Quarantine routines have been emphasized as a crucial factor in supporting sustainable parasite control and preventing the introduction of AR [19,33]. Farmers in Lithuania prefer to drench new sheep, similar to Norway [19], Sweden [21], and the Netherlands [34]. The trade of livestock both within and between countries appears to play a role in the spread of resistant parasites in Lithuania.

5. Conclusions

This study provides valuable insights into worm control practices on Lithuanian sheep farms, identifying key areas for improvement in combating gastrointestinal nematode infections. For instance, most respondents treated their sheep with anthelmintics routinely, without performing a prior diagnosis. Additionally, dose calculations were frequently based on visual estimates, which increases the risk of underdosing. The findings highlight the need for increased education on parasite management, especially for traditional farms. Emphasizing the use of diagnostic tools and the implementation of effective quarantine treatment protocols can help guide better treatment decisions and improve overall parasite control strategies. These measures will be essential in managing anthelmintic resistance and ensuring the long-term sustainability of sheep farming in Lithuania.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani15111542/s1, Questionnaire regarding gastrointestinal parasites in sheep farms in Lithuania.

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Abbreviations

The following abbreviations are used in this manuscript:

AR	Anthelmintic resistance
BZ	Benzimidazoles
CI	Confidence interval
GIN	Gastrointestinal nematodes
IVM	Ivermectin

LEV	Levamisole
ML	Macrocyclic lactones
MOX	Moxidectin
MDR	Multidrug resistance

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ACCESSORIES

Accessory No 1

Survey of Sheep Farmers

Farm ID:…………………….
Region: ……………………..…

1. What type of farm do you have?
   a. Traditional
   b. Organic
2. Please indicate the approximate number of animals

Sheep	Number
Yearlings/ewes	
Lambs	

3. What breed(s) of sheep do you raise?

4. Other animals kept

	Number		Number		Number
Goats		Cattle		Others	

5. Please indicate the approximate area of meadows and sown pastures used for sheep grazing:

	Area (ha)
a. Permanent pastures (not reseeded for more than 5 years)	
b. Rotational pastures (reseeded more often than every 5 years)	
c. Other (e.g., shrubs, forest edges, woodland)	
Total area	

6. Please indicate the type of water supply available in the pasture:
   a. Water troughs (water from a well or pipeline)
   b. Water troughs (water from a pond, lake, etc.)
   c. Open water bodies accessible to animals (pond, stream, lake)
   d. Other (please specify)
7. Please indicate the method of sheep grazing:
   a. Indoors (barn-based)
   b. Rotational grazing
   c. Grazing in the same pasture

   Approximate grazing start date:

   Approximate grazing end date:

8. Please indicate the main criteria you use to assess gastrointestinal worm infection:
   a. Fecal sample testing
   b. Data provided by slaughterhouses
   c. Diarrhea
   d. Poor quality/thin coat/wool
   e. Anemia
   f. Weight loss/low weight gain/milk yield
   g. Scientific literature and expert recommendations
   h. Veterinarian's recommendation
   i. Advice from another farmer or consultant
   j. Based on past experience

9. Do you periodically use anthelmintic drugs against parasites?

		Gastrointestinal nematodes	
		Yearlings/ewes	**Lambs**
Anthelmintic use	Yes (how many times per year)		
	No		

10. Which products do you use to treat gastrointestinal parasites?

Active ingredient	**Product names**	
Benzimidazole	(Panacur, Curaflukes)	
Levamisole	(Levamisol, Dehelman)	
Macrocyclic lactones	(Ivermektinas-Bimectin, Ivomec, ... / Doramektinas-Dectomax)	
Other		

11. Do you rotate anthelmintic products, and if so, which ones?

Yes	
No	

12. If you use anthelmintics for treatment, please indicate when this is done:
   a. Before mating/lambing/during dry period
   b. Immediately after lambing
   c. In spring, before turning out
   d. In spring, after turnout
   e. Before housing
   f. Before moving to a new pasture
   g. When clinical signs appear

13. How do you calculate the anthelmintic dose?
    a. Visual appraisal of weight
    b. Weighing a medium sized sheep
    c. Weighing the largest sheep
    d. Weigh each animal
    e. No treatment
14. When did you acquire the sheep (excluding local Lithuanian breeds)?
15. Where were the sheep acquired from?
16. Do you quarantine newly acquired animals?
    a. Yes
    b. No

Flock ID	N samples	**Strongylids**				***Strongyloides***				***Nematodirus***				***Trichuris***			
		Min	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**	**Min**	**Max**	**Mean**	**Median**
1	8	20	5400	1970	710	0	40	7.5	0	0	0	0	0	0	0	0	0
2	15	0	8120	1556	460	0	20	1.3	0	0	0	0	0	0	20	1.3	0
3	3	20	1260	573.3	440	20	160	73.3	40	0	0	0	0	0	0	0	0
4	11	0	2120	680	320	0	160	78	60	0	0	0	0	0	0	0	0
5	8	0	8060	817.8	300	0	300	35.6	0	0	80	5.6	0	0	0	0	0
6	15	100	420	198.7	200	0	200	33.3	0	0	0	0	0	0	20	1.3	0
7	3	120	420	223.3	190	0	60	20	10	0	0	0	0	0	0	0	0
8	11	0	880	213.3	160	0	300	71.1	0	0	0	0	0	0	0	0	0
9	8	40	200	107.3	120	0	40	14.5	20	0	0	0	0	0	0	0	0
10	15	60	300	150	120	0	0	0	0	0	0	0	0	0	0	0	0
11	3	20	220	115	110	0	520	170	80	0	20	5	0	0	20	5	0
12	11	0	3840	618	110	0	80	34	40	0	0	0	0	0	20	4	0
13	8	0	1280	296.7	90	0	80	15.6	0	0	80	5.6	0	0	0	0	0
14	15	0	180	86	80	0	40	8	0	0	0	0	0	0	0	0	0
15	3	0	1220	216.4	80	0	160	50.9	40	0	0	0	0	0	0	0	0
16	11	0	140	70	70	0	60	15	0	0	0	0	0	0	0	0	0
17	8	0	680	100	60	0	40	9.2	0	0	0	0	0	0	0	0	0
18	15	0	240	85	50	0	0	0	0	0	0	0	0	0	0	0	0
19	3	0	360	112.5	50	0	80	27.5	20	0	80	25	10	0	20	2.5	0
20	11	0	640	139	50	0	80	7	0	0	80	5	0	0	0	0	0

Flock ID	N samples	Strongylids				*Strongyloides*				*Nematodirus*				*Trichuris*			
		Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median
21	8	0	340	114.3	50	0	80	10	0	0	0	0	0	0	160	11.4	0
22	15	0	60	31.1	40	0	0	0	0	0	0	0	0	0	0	0	0
23	3	0	280	83.3	40	0	20	3.3	0	0	160	15	0	0	20	1.7	0
24	11	0	160	63.3	40	0	0	0	0	0	0	0	0	0	0	0	0
25	8	0	220	53.8	40	0	40	8.8	0	0	0	0	0	0	20	1.2	0
26	15	0	180	47.5	40	0	40	10	0	0	0	0	0	0	0	0	0
27	3	0	540	86.7	40	0	20	2.2	0	0	0	0	0	0	20	2.2	0
28	11	0	280	82	40	0	40	10	0	0	160	26	0	0	0	0	0
29	8	0	880	142	30	0	60	12	0	0	0	0	0	0	0	0	0
30	15	0	80	27.5	20	0	20	7.5	0	0	0	0	0	0	0	0	0
31	3	0	160	50	20	0	60	17.5	0	0	0	0	0	0	0	0	0
32	11	0	40	20	20	0	20	2.5	0	0	0	0	0	0	0	0	0
33	8	0	80	23.3	10	0	20	6.7	0	0	80	33.3	30	0	20	3.3	0
34	15	0	120	18.2	0	0	0	0	0	0	0	0	0	0	20	1.8	0
35	3	0	200	36.4	0	0	760	71.8	0	0	220	20	0	0	0	0	0
36	11	0	120	20	0	0	0	0	0	0	0	0	0	0	0	0	0
37	8	0	60	13.3	0	0	40	6.7	0	0	0	0	0	0	0	0	0
38	15	0	3500	258	0	0	0	0	0	0	0	0	0	0	20	1	0

CURRICULUM VITAE

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2015–2018	Veterinarian at L. Čėsnos firm Zoomedas
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