Dynamics of gastrointestinal nematode infections in sheep following treatment

Susmitha Chilamakuri

Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of
Animal sciences – Master´s programme
Uppsala 2026

Dynamics of gastrointestinal nematode infections in sheep following treatment

Author's name Susmitha Chilamakuri

Supervisor:	Giulio Grandi, SLU, Animal Biosciences (HBIO)
Assistant supervisor:	Johan Höglund, SLU, Animal Biosciences (HBIO)
Examiner:	Jean-Francois Valarcher, SLU, Dept of Clinical Sciences

Credits:	30 credits
Level:	A2E
Course title:	Independent project in Animal science, A2E
Course code:	EX0870
Programme/education:	Animal sciences Master´s programme
Course coordinating dept:	
Place of publication:	Uppsala
Year of publication:	2026
Copyright:	Example: All featured images are used with permission from the copyright owner.
Keywords:	Gastrointestinal nematodes, *Haemonchus contortus*, ddPCR, Anthelmintic resistance, Ivermectin, Fenbendazole, FECRT, Parasite monitoring.

Swedish University of Agricultural Sciences
Faculty of Veterinary Parasitology
Department of Animal Biosciences (HBIO)
Unit/section (add if needed or delete row)

Abstract

Gastrointestinal nematodes are a major constraint to sheep production due to their impact on animal health, productivity, and the increasing prevalence of anthelmintic resistance (AR). In this study, the efficacy of ivermectin (IVM) and fenbendazole (FBZ) was assessed using Fecal Egg Count Reduction Test (FECRT). In addition, the performance of an Artificial Intelligence (AI) assisted microscopy platform OvaCyte^{TM} (OC) was compared with droplet digital Polymerase Chain Reaction (ddPCR) for the detection and quantification of gastrointestinal nematodes in naturally infected Swedish lambs, with particular focus on *H. contortus.*

Twenty lambs 5-6 months old Gotlandic sheeps were randomly allocated to treatment with either IVM (0.2 mg/kg orally) or FBZ (5 mg/kg orally) and sampled at 1, 2, 3, and 4 weeks post-treatment, with a final follow-up sample collected six months later. Faecal samples were analysed using OC and ddPCR targeting the ITS2 region of ribosomal deoxyribonucleic acid (DNA). OC showed strong agreement with ddPCR for estimation of *H. contortus* abundance (Spearman's r = 0.77, $p < 0.0001$), although ddPCR demonstrated lower variability and greater sensitivity for the detection and quantification of *H. contortus*, particularly at low infection intensities.

Following a single treatment, IVM achieved >99% efficacy against all evaluated parasite groups throughout the monitoring period. In contrast, FBZ showed progressively reduced efficacy, particularly against trichostrongyles, *H. contortus*, and *Strongyloides* spp., indicating established benzimidazole resistance within the studied flock.

Overall, the findings demonstrate that OC provides a rapid and practically useful tool for flock-level parasite monitoring, while ddPCR remains the most analytically sensitive method for species-specific detection and confirmation of low-intensity infections. The combined application of AI-assisted microscopy and molecular

diagnostics may therefore support more evidence-based and sustainable parasite-control strategies in sheep production systems.

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Abbreviations

Abbreviation	Description
AI	Artificial Intelligence
AR	Anthelmintic resistance
EPG	Eggs Per Gram
FBZ	Fenbendazole
FECR	Faecal Egg Count Reduction
FECRT	Faecal Egg Count Reduction Test
GI	Gastrointestinal
Hc	*Haemonchus contortus*
IVM	Ivermectin
OC	Ovacyte™
REML	Restricted Maximum Likelihood
TST	Targeted Selective Treatment
WAAVP	World Association for the Advancement of Veterinary Parasitology

1. Introduction

Gastrointestinal parasitism represents a major constraint to small ruminant production worldwide (Elmahalawy et al. 2018), with significant implications for animal health, welfare, and economic sustainability. Among these, GI nematodes are particularly important due to their high prevalence, direct life cycles that facilitate efficient transmission between hosts without the need for intermediate hosts and capacity to cause both subclinical and clinical disease (Roeber et al. 2013; Blackie 2014). Infected animals may exhibit reduced growth performance, decreased feed efficiency, and increased susceptibility to secondary infections, resulting in substantial production losses (Roeber et al. 2013)

Among the nematodes affecting sheep, *Haemonchus contortus* is considered one of the most pathogenic species (Flay et al. 2022). As a hematophagous parasite residing in the abomasum, it is capable of causing severe anaemia, hypoproteinaemia, and, in acute infections, mortality (Cunha et al. 2024). Other nematodes, including *Teladorsagia circumcincta*, *Trichostrongylus* spp. and *Strongyloides papillosus*, also contribute to the overall parasite burden, particularly in mixed infections (Mavrot et al. 2015), where their combined effects can significantly impair host productivity.

Effective parasite control relies heavily on accurate and reliable diagnostic methods (Roeber et al. 2013a). Conventional coprological techniques, such as faecal egg count (FEC), are widely used due to their practicality and cost-effectiveness (Boareki et al. 2021). However, these methods are associated with several limitations, including high variability in egg shedding between individuals, overdispersion of faecal egg count data, and limited ability to differentiate morphologically similar parasite species (Boareki et al. 2021). These constraints reduce diagnostic precision and may compromise the effectiveness of parasite control strategies.

Recent technological advancements have led to the development of alternative diagnostic approaches. AI-based systems, such as automated image recognition

tools for faecal analysis (e.g. OC), offer rapid and standardized detection of parasite eggs, minimizing observer bias (Grandi et al. 2025). In parallel, molecular techniques such as ddPCR enable highly sensitive and specific detection of parasite DNA (Roeber et al. 2013), allowing accurate species-level identification even in low-intensity infections (Elmahalawy et al. 2018).

Despite these advances, the comparative performance and agreement between AI-based diagnostic tools and molecular methods remain insufficiently explored under practical conditions (Elghryani et al. 2025) (Grandi et al. 2025). In addition, accurate diagnostic techniques are essential not only for parasite detection but also for evaluating anthelmintic efficacy and monitoring the emergence of AR in sheep production systems (Roeber et al. 2013). A better understanding of the strengths and limitations of these approaches is essential for their integration into parasite monitoring and control programs.

The aim of this study was twofold: i) to assess the efficacy of the two most widely used anthelmintic drugs against GI nematodes in sheep in Sweden, and ii) to evaluate the performance of an AI-assisted microscopy platform (OC) compared with a validated ddPCR assay for detection and quantification of *H. contortus*. By directly comparing these two diagnostic methods, the study aims to determine whether AI-driven microscopy can achieve analytical sensitivity and quantitative reliability comparable to ddPCR while offering potential advantages in accessibility and practical implementation. This comparison assesses the feasibility of integrating AI-based diagnostics into parasite surveillance frameworks and contributes to the development of accurate, scalable, and evidence-based strategies for precision parasite control in livestock production systems.

2. Literature study

2.1 Host–parasite interactions and distribution dynamics

GI parasitism in ruminants is governed by a dynamic interaction between parasites, hosts, and the environment (Bricarello et al. 2023). From an evolutionary perspective, a balance exists in which parasites are able to survive and reproduce without causing excessive harm, while hosts have developed physiological and behavioural mechanisms such as selective grazing (Loon et al. 2002) to limit parasite burden. This balance can be disrupted by increased host susceptibility (e.g., stress, immunosuppression, age, or genetic factors) or elevated infection pressure associated with environmental and management conditions, including high stocking density and favourable climatic factors (O'Connor et al. 2006).

Parasitic infections are typically aggregated within host populations, with a small proportion of individuals harbouring the majority of parasites (Shaw & Dobson 1995). This uneven distribution results in considerable variation in parasite burden and egg excretion, which has important implications for transmission dynamics and diagnostic interpretation. Even subclinical infections (Mavrot et al. 2015) can negatively affect host performance by reducing feed intake, growth, and reproductive efficiency. In addition, polyparasitism where multiple parasite species coexist within the same host may further exacerbate health impacts, particularly in combination with environmental stressors or co-infections (Ribeiro 2015).

2.2 Gastrointestinal helminths and pasture-borne transmission

GI parasites of ruminants are primarily composed of helminths, which are classified into cestodes (tapeworms), trematodes (flukes), and nematodes (roundworms) (Taylor et al. 2015). Among these, nematodes are of particular importance in grazing systems due to their direct life cycles and efficient transmission via pasture (Deplazes et al. 2016).

Most GI nematodes exhibit a direct life cycle involving both parasitic and free-living stages. Adult worms in the GI tract produce eggs that are excreted in faeces and develop in the environment through successive free living larval stages (L1–L3). The infective third-stage larvae (L3) are ingested during grazing, after which they develop into adult worms within the host, completing the life cycle (O'Connor et al. 2006).

Transmission is therefore highly dependent on environmental conditions, particularly temperature and moisture, which influence larval development and survival. Direct host-to-host transmission is uncommon, as parasites generally require a period of external development before becoming infective (Deplazes et al. 2016).

Based on this background, the following sections present key pasture-borne nematodes of ruminants, with emphasis on their epidemiology, life cycle, and relevance for the host.

Table 1.Taxonomic classification and key biological characteristics of selected gastrointestinal nematodes of ruminants.

Order	Family	Genus	Intermediate Host	Main Location in Host	Animals Affected
Strongylida	Trichostrongylidae	*Haemonchus* (*H. contortus*, *H. placei*)	None	Abomasum	Sheep, goats, cattle
		Teladorsagia	None	Abomasum (sometimes duodenum)	Sheep, goats
		Trichostrongylus	None	Abomasum and/or small intestine	Sheep, goats, cattle
		Cooperia	None	Small intestine (occasionally abomasum)	Cattle, sheep, goats
		Nematodirus	None	Small intestine	Sheep, goats, cattle
Rhabditida	Strongyloididae	*Strongyloides*	None (free-living and parasitic generations)	Small intestine	Cattle, sheep, pigs, horses, dogs, cats, humans (species-dependent)

2.3 Trichostrongyloidea

The superfamily *Trichostrongyloidea* includes the most clinically and economically significant gastrointestinal nematode genera of ruminants, including *Haemonchus*, *Trichostrongylus*, *Cooperia*, *Teladorsagia*, and *Nematodirus* (Taylor et al. 2015). All have a direct life cycle, in which eggs excreted in faeces develop into infective third-stage larvae (L3) on pasture and are ingested during grazing (Jacobs et al. 2016).

A key feature of trichostrongyles is that L3 larvae retain the cuticle of the previous stage (L2), forming a protective sheath that enhances environmental

survival but limits feeding (Deplazes et al. 2016). Transmission is therefore highly dependent on environmental conditions, particularly temperature and moisture, which influence larval development, migration onto herbage, and survival. In temperate regions, larvae may overwinter, contributing to seasonal infection patterns.

In sheep, infections are typically mixed and aggregated, with a small proportion of animals harbouring most parasites. Many species can undergo arrested development (hypobiosis) (Taylor et al. 2016), allowing larval stages to persist within the host during unfavourable conditions. Pathogenic effects depend on parasite location, with abomasal species affecting gastric function and intestinal species impairing nutrient absorption, leading to reduced productivity even in subclinical infections (Taylor et al. 2016).

2.3.1 Haemonchus

Haemonchus contortus (*Hc*) is a highly pathogenic abomasal nematode. Following ingestion of L3 larvae, it develops in the abomasum, where both larval and adult stages feed on blood. This can result in significant blood loss (approximately 0.05 ml per adult worm per day) and severe anaemia (Jacobs et al. 2016).

The parasite may undergo hypobiosis at the L4 stage, enabling survival under adverse conditions (Taylor et al. 2015). In sheep, infection leads to haemonchosis, characterized by anaemia, weakness, submandibular oedema, and in severe cases sudden death, typically without diarrhoea.

2.3.2 Teladorsagia

Teladorsagia circumcincta is a common gastrointestinal nematode of sheep and a major cause of parasitic gastroenteritis and associated production losses important in temperate sheep-producing regions, including Sweden (Taylor et al. 2016). The parasite inhabits the abomasum, where sheep become infected through ingestion of infective third-stage larvae (L3) from contaminated pasture. Developing larvae and adult worms compromise the integrity of abomasal mucosa, impairing digestion

and reducing growth and productivity, particularly in lambs and heavily infected grazing animals (Deplazes et al. 2016). The parasite may also undergo hypobiosis during unfavourable environmental conditions (Taylor et al. 2016).

2.3.3 Trichostrongylus

The genus *Trichostrongylus* includes several species that infect either the small intestine or the abomasum and are transmitted via ingestion of L3 larvae from pasture (Taylor et al. 2015). In sheep, they are associated with chronic parasitic gastroenteritis, causing villous damage, diarrhoea, and reduced weight gain (Deplazes et al. 2016). Although less pathogenic on their own, they contribute significantly to disease in mixed infections.

2.3.4 Cooperia

Cooperia spp. are small intestinal nematodes with a similar direct life cycle to other trichostrongyles (Taylor et al. 2015). In sheep, they are generally of low pathogenicity but may contribute to reduced growth and feed efficiency, particularly in young animals and mixed infections. Their importance is increasing due to their association with AR (Bartley et al. 2012).

2.3.5 Nematodirus

Nematodirus spp. are intestinal nematodes characterized by development of the infective L3 stage within the egg (Jacobs et al. 2016). Hatching requires specific environmental cues, typically a period of cold followed by warming (Taylor et al. 2015).

In sheep, *Nematodirus battus* is particularly important, as its resistant eggs allow synchronized hatching and sudden infection peaks in lambs (Deplazes et al. 2016). Infection can cause severe diarrhoea and mortality, often before eggs are detectable in faeces.

2.4 Strongyloides

Unlike the previous the genus, *Strongyloides* belongs to the superfamily *Rhabditoidea* and these nematodes differs from trichostrongyles by having a life cycle that includes alternating parasitic and free-living generations (Taylor et al. 2015). In sheep, the dominant species is *S. papillosus*. The parasitic phase occurs in the small intestine and consists only of female worms, which reproduce by parthenogenesis and produce eggs that are excreted larvated in the faeces.

A key feature is the ability of larvae to develop either directly into infective third-stage larvae (L3) or into a free-living generation, allowing rapid multiplication in the environment. Transmission occurs via skin penetration, oral ingestion, or lactogenic routes, followed by systemic migration through the lungs before establishment in the intestine (Deplazes et al. 2016).

In sheep, *S. papillosus* mainly affects young animals, where it may cause diarrhoea, reduced growth, and occasionally mortality. Development is favoured by warm and humid conditions, while infections in adults are typically subclinical but contribute to transmission (Taylor et al. 2015).

3. Diagnosis

3.1 Faecal egg counts

FEC methods are the most widely used diagnostic approach for GI nematode infections in ruminants. These techniques estimate parasite burden by quantifying the number of eggs per gram (EPG) of faeces, typically using methods such as the McMaster technique. FEC is widely applied due to its simplicity, cost-effectiveness (Boareki et al. 2021), and suitability for routine field use, and it remains the standard method for monitoring parasite infections and assessing anthelmintic efficacy through faecal egg count reduction tests (FECRT) (Coles et al. 1992).

Despite its widespread use, FEC has several important limitations. Egg excretion is biologically variable and typically over dispersed, with a small proportion of animals heavily contributing to pasture contamination. In addition, egg counts do not always correlate directly with worm burden, and morphological similarity between strongyle-type eggs limits their identification at the species level. The method is also operator-dependent, with variability introduced by sample preparation, flotation techniques, and interpretation (Roeber et al. 2013). Furthermore, low-intensity or prepatent infections may not be detected, leading to underestimation of parasite presence.

3.2 Artificial intelligence -based image analysis

Artificial intelligence-based image analysis systems, such as OC (Telenostic^{Ltd}, Ireland), have been developed to improve the objectivity and efficiency of faecal egg count diagnostics (Grandi et al. 2025). These systems combine digital microscopy with machine learning algorithms to automatically detect and quantify parasite eggs based on morphological features (Elghryani et al. 2026). By applying consistent classification criteria, AI-based methods reduce operator-dependent variability and improve standardisation compared to conventional microscopy – including long-term access to captured scans. In addition, automated analysis enables rapid processing of large sample numbers, supporting their application in

flock-level monitoring and targeted selective treatment strategies (Grandi et al. 2025; Elghryani et al. 2026).

Despite these advantages, several limitations remain. The performance of AI-based systems depends on the quality and representativeness of the training data, and variability in sample preparation or egg morphology may affect detection accuracy (Litjens et al. 2017; Grandi et al. 2025). Furthermore, as with traditional coprological methods, detection relies on the presence of eggs in faeces and may therefore underestimate infections with low or intermittent egg shedding. Consequently, validation against more sensitive reference methods, such as molecular diagnostics, is required to assess their reliability under field conditions (Elmahalawy et al. 2018; Grandi et al. 2025; Elghryani et al. 2026).

3.3 Molecular diagnostics

Molecular diagnostic techniques, such as ddPCR, have emerged as highly sensitive and specific tools for the detection of GI nematodes (Elmahalawy et al. 2018; Baltrušis & Höglund 2023). The ddPCR technology is based on the partitioning of a sample into thousands of nanolitre-sized droplets, each acting as an independent amplification reaction. Following PCR amplification, droplets are counted, classified as positive or negative, and the absolute concentration of target DNA is calculated using Poisson statistic (Pinheiro et al. 2012). This approach eliminates the need for standard curves and reduces dependence on amplification efficiency (Hindson et al. 2011).

A key advantage of ddPCR is its ability to detect low-abundance parasite DNA, allowing identification of infections that may not be detectable by conventional faecal egg count methods, particularly in cases of low or intermittent egg shedding (Pinheiro et al. 2012; Roeber et al. 2013; Elmahalawy et al. 2018). In addition, depending on the assay design, ddPCR enables species-specific detection, overcoming limitations associated with the morphological similarity of strongyle-type eggs (Elmahalawy et al. 2018). The method also shows increased tolerance to PCR inhibitors commonly present in faecal samples, improving analytical reliability (Taly et al. 2013).

However, ddPCR requires specific assay design, specialised equipment, technical expertise, and controlled laboratory conditions, which may limit its routine application in large-scale or field-based settings (Baltrušis & Höglund 2023). Despite these limitations, ddPCR represents a highly reliable reference method for parasite detection and is particularly valuable for validation of alternative diagnostic approaches.

4. Anthelmintic treatments and resistance

The control of GI nematodes in sheep relies heavily on the use of anthelmintic drugs. However, the increasing prevalence of AR has become a major global concern (Kaplan 2004), including in Europe (Rose Vineer et al. 2020), where reduced efficacy of multiple drug classes has been widely reported. Consequently, GI nematodes are no longer considered solely a manageable production constraint but represent a significant and growing threat to the sustainability of small ruminant farming and animal health (Kaplan 2004; Huang et al. 2016).

Within this global context, Sweden faces similar challenges. Although it has a temperate climate, environmental conditions during spring and summer favour the development and transmission of infective larvae on pasture (Höglund et al. 2022). As sheep production in Sweden is largely pasture-based, grazing animals are continuously exposed to infective stages during the grazing season (Höglund et al. 2022). Reports of AR in Swedish sheep flocks further emphasise that parasite control is an ongoing and increasing challenge at the national level (Rose Vineer et al. 2020b; Höglund et al. 2022).

In Sweden, the availability of anthelmintic drugs for use in sheep is relatively limited. Benzimidazoles (BZs) and ivermectin (IVM) represent the main single-active substances licensed for routine use, while other compounds such as levamisole and monepantel are generally reserved for cases where reduced efficacy of first-line treatments has been identified, for example through FECRT (Höglund et al. 2020; 2022). This limited availability further emphasises the importance of preserving drug efficacy.

In response to increasing resistance, parasite control strategies have shifted towards evidence-based approaches such as targeted selective treatment (TST), in which only animals exceeding a defined infection threshold are treated. This approach aims to reduce selection pressure for resistance while maintaining adequate parasite control (Maciel et al. 2009; Reck et al. 2014). However, the effectiveness of TST depends on accurate and reliable diagnostic methods (Maciel

et al. 2009). Inadequate sensitivity or variability in diagnostic tools may lead to incorrect treatment decisions, either by failing to treat infected animals or by unnecessarily treating low-risk individuals, thereby accelerating resistance development. Therefore, robust and dependable diagnostics are essential to support sustainable parasite control strategies (Reck et al. 2014).

4.1 Ivermectin

IVM is a member of the macrocyclic lactone group, specifically the avermectins, and is widely used in the control of GI nematodes in sheep (Jacobs et al. 2016). Macrocyclic lactones act by binding to glutamate-gated chloride ion channels in the parasite nervous system, leading to increased chloride influx, paralysis, and eventual death of the parasite (Taylor et al. 2016).

IVM exhibits broad-spectrum activity against a wide range of nematodes and is also effective against certain ectoparasites. However, it has limited or no activity against trematodes and cestodes. Despite its effectiveness, resistance to IVM has been increasingly reported in GI nematodes, particularly in *H. contortus*, which has significant implications for parasite control in sheep (Taylor et al. 2016).

4.2 Fenbendazole

FBZ belongs to the benzimidazole class of anthelmintics, which also includes compounds such as albendazole and oxfendazole (Deplazes et al. 2016). Benzimidazoles act by binding to β-tubulin in parasite cells, disrupting microtubule formation and inhibiting glucose uptake, ultimately leading to energy depletion and death of the parasite.

FBZ is widely used in sheep due to its broad-spectrum activity against GI nematodes and its relatively low toxicity. However, resistance to benzimidazoles is now widespread in many nematode populations, particularly in *H. contortus*, reducing treatment efficacy and complicating parasite control strategies (Taylor et al. 2016).

5. Materials and Methods

5.1 Sample collection and anthelmintic treatments

The study involved twenty lambs, aged in between 5-6 months at the start of the experiment, naturally infected with GI nematodes. All animals were from a flock in outside Uppsala in central Sweden (farm_size: 130 ewes, Gotlandic pelt sheep) and were managed under similar husbandry and grazing conditions throughout the experimental period to minimise environmental and nutritional variation.

The sheep were randomly allocated to two equal treatment groups, each comprising ten animals at the start of the experiment. One group (group I, I1-I10) received IVM orally (Noromectin vet 0.8 mg/ml, Norbrook, Sweden), while the second group (group F, F1-F10) received FBZ (Axilur vet 2,5%, MSD Animal Health, Sweden) at the recommended doses (0.2 mg/kg for IVM and 5 mg/kg for FBZ).

Individual faecal samples were collected from all twenty sheep before anthelmintic treatment on 24 September 2025, designated as time point zero (T0). These baseline measurements were used to assess pre-treatment infection intensity and estimate treatment efficacy during the first weeks after treatment.

Post-treatment faecal sampling was conducted longitudinally to monitor parasite dynamics and evaluate anthelmintic efficacy over time. Samples were collected from the same individuals (I1–I10 for the IVM-treated group and F1–F10 for the FBZ-treated group) at five time points following treatment. Sampling was performed at approximately one-week intervals, with collections on 1 October 2025 (T1), 7 October 2025 (T2), 15 October 2025 (T3), 21 October 2025 (T4), and a final time point on 24 March 2026 (T5). At the final sampling point (T5), some animals were no longer available due to sale, resulting in missing samples from five animals: I3 and I6 in the IVM group and F2, F3, and F10 in the FBZ group. Despite these losses, longitudinal data were retained for the remaining animals to assess treatment response over time.

At each time point, individual faecal samples were collected directly from the rectum of each animal using clean disposable gloves to avoid cross-contamination. Samples were placed in individually labelled containers, maintained at 4 °C during transport, and delivered to the laboratory within approximately 2 h for further analysis.

All animal-related activities were approved by the Uppsala Animal Ethics Committee (permit no. Dnr 5.8.18–12,184/2023) and conducted in accordance with Swedish animal welfare legislation.

5.2 Sample preparation

Faecal samples were prepared strictly in accordance with the manufacturer's instructions as outlined in the OC Equine and Large Animal Standard Faecal Egg Count preparation protocol (Telenostic Ltd, Ireland). A total of 115 faecal samples were processed for analysis. The materials required for sample preparation, as specified by the manufacturer, included a digital scale, plastic specimen containers, a metal sieve, flotation solution, a homogeniser, a spatula, a syringe, a pipette, and OC cassettes. Before processing, each sample was assigned a unique identification number and registered in the OC software to ensure full traceability throughout the analytical workflow.

Following the manufacturer's recommended procedure, an empty plastic container was placed on a calibrated digital scale and tared to zero. Three grams of faeces were weighed using a spatula, after which forty-seven grams of flotation solution (corresponding to around 41.5 ml) with a specific gravity of 1.200, supplied by the manufacturer, were added to achieve the prescribed dilution ratio. The mixture was thoroughly homogenised to ensure even distribution of parasite eggs within the suspension and to maintain consistency across samples.

In accordance with the protocol, the homogenised suspension was filtered through a metal sieve with a mesh size of 0.25 mm, a diameter of 52 mm, and a height of 18 mm into a clean beaker to remove larger debris. The sieve was then

removed, and the filtrate was immediately aspirated using a syringe to minimise egg flotation before analysis.

Approximately eight millilitres of the prepared suspension were degassed to eliminate air bubbles and slowly injected into the OC cassette. During filling, the cassette was held vertically with the adhesive label at the twelve o'clock position, and the suspension was carefully introduced through the designated inlet port until the chamber was filled. Once loaded, the cassette was transferred to the AI-based OC microscope and securely fastened to ensure stable positioning during scanning.

The analyses were performed using the OC Ovine Plus/Speciation model in standard mode. Approximately 2.33 mL of sample suspension was analysed, generating around 92 microscopic images per sample. Egg counts were converted to EPG using the Ovacyte calibration factor of 7, which accounts for the dilution ratio and analysed sample volume, such that each detected egg corresponds to approximately 7 EPG.

Figure 1. Preparation and loading of an OvacyteTM cassette with homogenised faecal suspension prior to automated image analysis. Photo credit: Susmitha Chilamakuri

The device captured multiple high-resolution microscopic images of the sample, which were analysed using an integrated AI algorithm trained to recognise parasite eggs based on their morphological characteristics, including size, shape, and structural features. The system automatically distinguished parasite eggs from background debris and other particulate matter, enabling objective and standardised

enumeration without manual counting. Before image acquisition, the instrument performed internal mixing/succussion and calibration procedures to facilitate the flotation process within the cassette and to ensure consistency of analysis. Results were generated by the software and expressed as eggs per gram of faeces. This model allows the differentiation among different egg types, for example strongyles, trichostrongyles, *Haemonchus contortus*, *Strongyloides* sp.

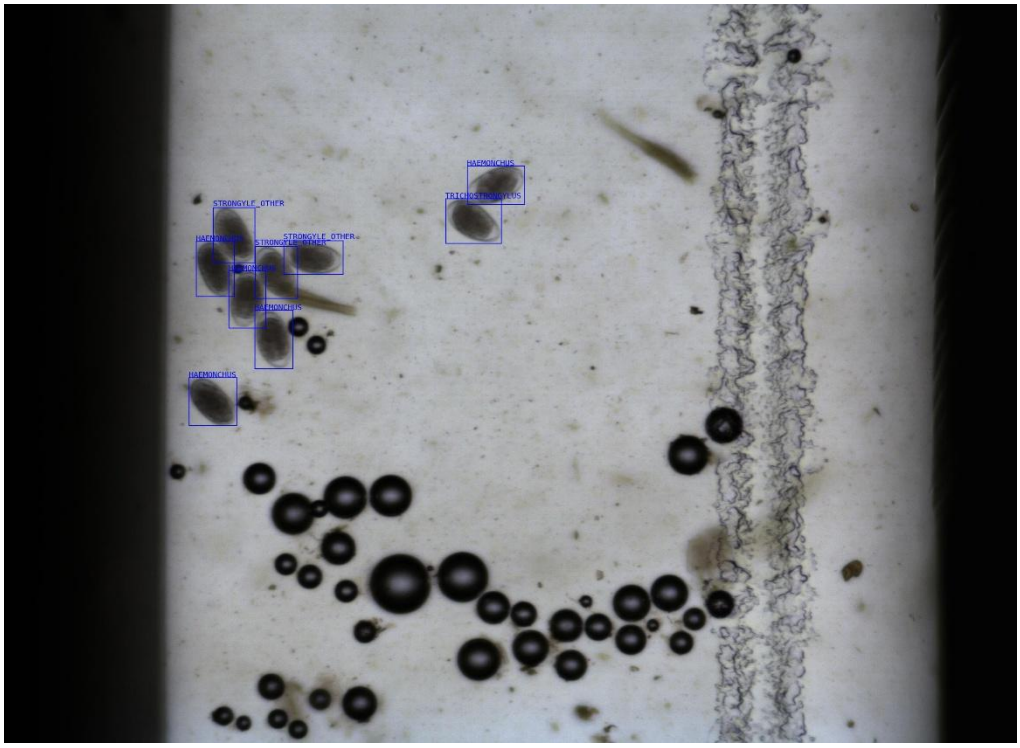

Figure 2. OvacyteTM showing automated detection and classification of gastrointestinal nematode eggs in a sheep faecal sample.

5.3 Post Scanning Processing

Following OC scanning, the liquid content of each cassette was carefully recovered by repeatedly rinsing the cassette with tap water to ensure complete collection of the sample suspension. The recovered liquid was transferred into a 50 mL centrifuge tube and adjusted to a final volume of ≈50 mL with tap water. The suspension was then homogenised by centrifugation at 1000 g for 5 minutes.

After centrifugation, the supernatant was carefully discarded, leaving the sediment at the bottom of the tube. The sediment was gently resuspended by

repeated pipetting to ensure uniform mixing. The concentrated sediment was subsequently transferred into labelled microcentrifuge tubes and centrifuged for 15000 g for 5 minutes to remove excess liquid from the samples. This step ensured that residual water was eliminated prior to further processing, allowing more efficient DNA extraction and preventing dilution of reagents in subsequent ddPCR reactions. The processed samples were subsequently stored at −18 °C until further use.

Prior to DNA extraction, all recovered sediment samples were weighed to ensure compliance with the manufacturer's recommended input range. A total of 115 samples were measured and recorded. Sample weights varied considerably, ranging from 150 mg to 750 mg. According to the DNA extraction kit protocol, an optimal input of 180–220 mg of material is required.

To evaluate whether the protocol would remain effective across different sediment volumes, a preliminary test was conducted using three representative samples. These test samples were prepared following the same flotation and filtration procedure described previously. Briefly, three grams of faeces were homogenised with forty-seven grams of flotation solution (specific gravity 1.200), filtered through a 0.25 mm mesh metal sieve into a beaker, and the resulting suspension was collected.

From the filtered suspension, three different aliquot volumes (8 mL, 15 mL, and 35 mL) were transferred into separate 50 mL centrifuge tubes. Each tube was then brought to a final volume of 50 mL with tap water and homogenised by centrifugation at 1,500 rpm for 3 minutes. Following centrifugation and removal of the supernatant, the resulting sediments were weighed, yielding 0.195 g for the 8 mL aliquot, 0.276 g for the 15 mL aliquot, and 0.357 g for the 35 mL aliquot. These samples were subsequently processed to evaluate the suitability of sediment yield for DNA extraction.

DNA extraction was initially performed strictly according to the manufacturer's protocol (NucleoSpin® DNA Stool). However, during the precipitation contaminants step, centrifugation at 13,000 × g for 3 minutes did not consistently

yield the recommended volume of approximately 600 µL of supernatant. Due to insufficient recovery, optimisation trials were conducted to improve sample yield.

First, the centrifugation speed was increased to 15,000 × g for 3 minutes, which resulted in improved supernatant recovery suitable for downstream processing. After transferring the supernatant, 100 µL of Buffer ST2 was added as specified in the protocol, and samples were incubated as recommended. The second centrifugation step was then further optimised by increasing the speed from 13,000 × g to 20,000 × g for 3 minutes to enhance pellet formation and lysate clarification.

These experimental modifications were implemented in response to initial protocol limitations and successfully improved sample recovery for DNA purification.

Genomic DNA was extracted from concentrated faecal sediment using the NucleoSpin DNA Stool kit (Macherey-Nagel, Germany) in according to the manufacturer's instructions, with the abovementioned optimisation steps implemented after preliminary testing to improve sample recovery.

Faecal sediment samples recovered from the OC cassettes were thawed at room temperature before DNA extraction. After thawing, 850 µL of Buffer ST1 was added to each sample along with the provided bead matrix to facilitate disruption of parasite eggs and cellular material. The samples were then incubated at 70 °C for 5 minutes to promote initial cell lysis and soften particulate debris. Following heat treatment, homogenisation was performed using a Precellys Evolution Touch (Bertin Technologies, France) at 10,000 rpm for five cycles of 60 seconds, with 20-second intervals between cycles to prevent overheating. This combined thermal and mechanical treatment ensured efficient disruption of parasite eggs and thorough homogenisation of the sediment within the lysis buffer.

The homogenised lysates were centrifuged at 15,000 × g for 3 minutes to pellet insoluble debris and particulate matter. After centrifugation, approximately 600

µL of the clear supernatant containing released DNA was carefully transferred into fresh sterile 2 mL microcentrifuge tubes, ensuring the pellet remained undisturbed.

To the supernatant, 100 µL of Buffer ST2 was added to promote precipitation of contaminants and PCR inhibitors commonly present in faecal samples. The tubes were gently vortexed for approximately 5 seconds to ensure thorough mixing, then incubated at 2–8°C for 5 minutes to enhance inhibitor precipitation. After incubation, the samples were centrifuged at 20,000 × g for 3 minutes to pellet the precipitated contaminants.

Approximately 550 µL of the clarified supernatant was transferred to a NucleoSpin® Inhibitor Removal Column (red ring) placed in a 2 mL collection tube with lid. The column was centrifuged at 13,000 × g for 1 minute to filter the lysate and remove inhibitory substances. After centrifugation, the inhibitor removal column was discarded, while the filtered lysate remaining in the collection tube was retained for subsequent DNA binding steps.

After lysate filtration, the binding conditions were adjusted by adding 200 µL of Buffer ST3 to the filtered lysate. The tube was securely closed and briefly vortexed for approximately 5 seconds to ensure thorough mixing and optimal conditions for DNA binding to the silica membrane in the next purification step.

To bind the DNA, a NucleoSpin® DNA Stool Column (green ring) was placed in a 2 mL collection tube. Up to 700 µL of the sample was carefully loaded onto the column and centrifuged at 13,000 × g for 1 minute to allow DNA adsorption onto the silica membrane. The flow-through was then discarded, and the column was returned to the same collection tube for further purification.

Following DNA binding, the silica membrane of the NucleoSpin® DNA Stool Column (green ring) was purified through a series of wash steps to remove residual contaminants and inhibitory substances.

For the first wash, 600 µL of Buffer ST3 was carefully added directly onto the membrane of the NucleoSpin® DNA Stool Column positioned in a 2 mL collection tube. The column was centrifuged at 13,000 × g for 1 minute. After centrifugation,

the flow-through was discarded, and the NucleoSpin® DNA Stool Column was placed back into the same collection tube.

For the second wash, 550 µL of Buffer ST4 was applied to the membrane of the NucleoSpin® DNA Stool Column and centrifuged at 13,000 × g for 1 minute. The flow-through was discarded, and the column was returned to the same collection tube.

For the third wash, 700 µL of Buffer ST5 was added to the NucleoSpin® DNA Stool Column. The lid was securely closed, and the column was briefly vortexed for approximately 2 seconds to ensure uniform contact of the wash buffer with the membrane. The column was then centrifuged at 13,000 × g for 1 minute. The flow-through was discarded, and the column was placed back into the same collection tube.

For the fourth and final wash, 700 µL of Buffer ST5 was added to the membrane of the NucleoSpin® DNA Stool Column, followed by centrifugation at 13,000 × g for 1 minute. The flow-through was discarded, and the column was returned to the same collection tube to complete the washing procedure.

After the final wash, the NucleoSpin® DNA Stool Column underwent an additional dry centrifugation at 13,000 × g for 2 minutes to remove residual wash buffer and ensure complete drying of the silica membrane. If residual liquid remained in contact with the column, an additional centrifugation step was performed under the same conditions.

For DNA elution, the NucleoSpin® DNA Stool Column was transferred into a clean 1.5 mL microcentrifuge tube. Depending on the desired balance between for DNA concentration and total yield, Buffer SE was added directly to the centre of the silica membrane, with recommended volumes of 30 µL for high DNA concentration, 50 µL for moderate concentration and yield, or 100 µL for maximum yield. In this study, 50 µL of Buffer SE was used to achieve an optimal balance between DNA concentration and yield suitable for downstream ddPCR analysis.

After addition of the elution buffer, the column lid was closed, and the sample was centrifuged at 13,000 × g for 1 minute to elute the DNA into the collection tube. The NucleoSpin® DNA Stool Column was then discarded, and the eluted DNA was briefly vortexed for approximately 2 seconds to ensure homogeneity. Each tube was subsequently labelled and stored at −20°C until further molecular analysis.

Figure 3. Precellys Evolution Touch homogenizer used for bead-beating lysis.

5.4 Droplet digital PCR

Droplet digital PCR was performed using *H. contortus*-specific and universal strongylid (UNI) primer/probe sets targeting different regions of the ribosomal RNA gene array (Elmahalawy et al. 2018) . Absolute DNA copy numbers obtained from the two assays were subsequently used to estimate the proportion of *H. contortus* within the total strongylid nematode population. Each reaction was prepared in a final volume of 22 µL, consisting of 11 µL of 2 × ddPCR Supermix for probes, 1.1 µL of universal primer/probe mix, 1.1 µL of Hc-specific primer/probe mix, 2 µL of template DNA, and 6.8 µL of molecular-grade water. The reaction mixtures were partitioned into approximately 20,000

nanolitre-sized droplets using a Bio-Rad droplet generator and then transferred to a 96-well plate. PCR amplification was carried out under the following conditions: initial enzyme activation at 95°C for 10 min, followed by 40 cycles of denaturation at 94°C for 30 s and annealing/extension at 57°C for 1 min, and a final enzyme deactivation step at 98°C for 10 min with a ramp rate of 2°C/s. Following amplification, droplets were analysed using the Bio-Rad QX200 Droplet Digital PCR system, and fluorescence data were processed using QuantaSoft™ analysis software (also provided by Bio-Rad) to distinguish positive and negative droplets based on threshold setting of 4000. Absolute DNA concentrations were calculated as copies per µL using Poisson statistics.

Initial experiments using undiluted DNA samples from both the IVM and FBZ groups at time point T0 resulted in signal saturation, indicating that the DNA concentration exceeded the assay's optimal dynamic range. Therefore, a 1:100 dilution was applied to T0 samples to enable accurate quantification. Samples at time point T1 were analysed undiluted for both groups. For time points T2, T3, and T4, samples from the F group a 1:10 was required, while samples from the I group were analysed undiluted. At time point T5, all samples from both groups were analysed undiluted. This dilution strategy – followed by the normalization of copy numbers - ensured reliable quantification across samples with varying DNA concentrations while minimising droplet saturation effects.

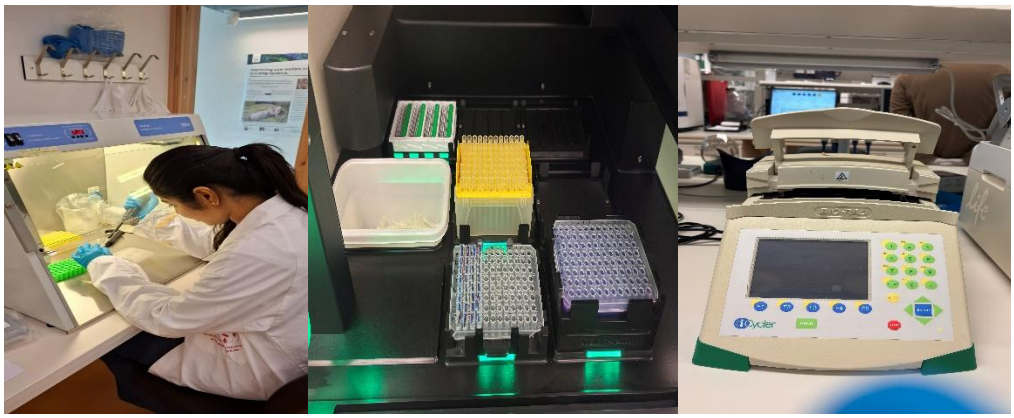

Figure 4.Sequential workflow of ddPCR analysis showing preparation of ddPCR reaction plates, droplet generation, and PCR amplification using a thermal cycler.

5.5 Statistical analysis

5.5.1 Faecal egg counts

Egg counts (x+1) were log-transformed before analysis to approximate a normal distribution. Analyses were conducted using a mixed-effects model fitted by restricted maximum likelihood (REML), with time (T0 to T4) included as a repeated measure and treatment group (FBZ vs IVM) as a fixed effect. The interaction between time and treatment was also evaluated. Where appropriate, a Geisser–Greenhouse correction was applied to account for violations of sphericity.

Post hoc multiple comparisons were performed using Šídák's correction to adjust for multiple testing. Pairwise comparisons were made between treatment groups at each time point and within treatment groups across time points.

All analyses were conducted using GraphPad Prism version 11 for macOS. Results are presented as mean differences with 95% confidence intervals. Adjusted p-values < 0.05 were considered statistically significant.

5.5.2 Proportion of *H. contortus*

The proportion of *H. contortus* (%Hc) was calculated by dividing the *H. contortus*-specific DNA copy number by the total strongylid DNA copy number obtained using the universal (UNI) assay. The relationship between % Hc estimated by OC and ddPCR was assessed using Spearman's rank correlation coefficient (r), due to the non-normal distribution of the data. The strength and direction of the association were evaluated, and 95% confidence intervals were calculated. Statistical significance was determined using a two-tailed test, with $p < 0.05$ considered significant.

5.5.3 Faecal egg counts reductions

Faecal egg count reduction (FECR) was estimated using paired pre- and post-treatment faecal egg count (FEC) data obtained from the same animals. This study design is consistent with the recommendations of the World Association for the

Advancement of Veterinary Parasitology (WAAVP), which recognize paired sampling as an approach that improves the accuracy and sensitivity of FECRT analysis.

Statistical analysis of FECR was performed using a Shiny-based analytical model, which provided FECR estimates for each parasite group together with associated uncertainty intervals. These intervals represent the range of plausible values for the true FECR and reflect both the variability in faecal egg counts between animals and the precision of the model estimates.

6. Results

6.1 Faecal egg counts

Egg counts of strongyles (Fig 5A) and trichostrongyles (Fig 5B) were significantly affected by time, treatment, and their interaction (strongyles: $F(3.22, 58.0) = 72.9$, $p < 0.0001$; $F(1, 18) = 20.8$, $p = 0.0002$; $F(3.22, 58.0) = 13.0$, $p < 0.0001$), with both parasite groups showing similar temporal patterns. No differences were observed at baseline, but counts declined following treatment, with a markedly greater reduction in the IVM-treated group, approaching zero from T1 onwards, whereas the FBZ group showed a more gradual decrease. Post hoc comparisons confirmed significantly lower counts in the IVM group at all post-treatment time points ($p \leq 0.0178$). Overall, mean counts were lower in the IVM group (mean difference = 0.982, 95% CI: 0.530–1.43).

A similar pattern was observed for *H. contortus* (Fig 5C) and *Strongyloides* sp. (Fig 5D), both showing significant effects of time, treatment, and their interaction (all $p \leq 0.0124$). Egg counts declined over time in all groups but were consistently lower in IVM-treated animals, indicating a stronger and more persistent treatment effect compared to FBZ.

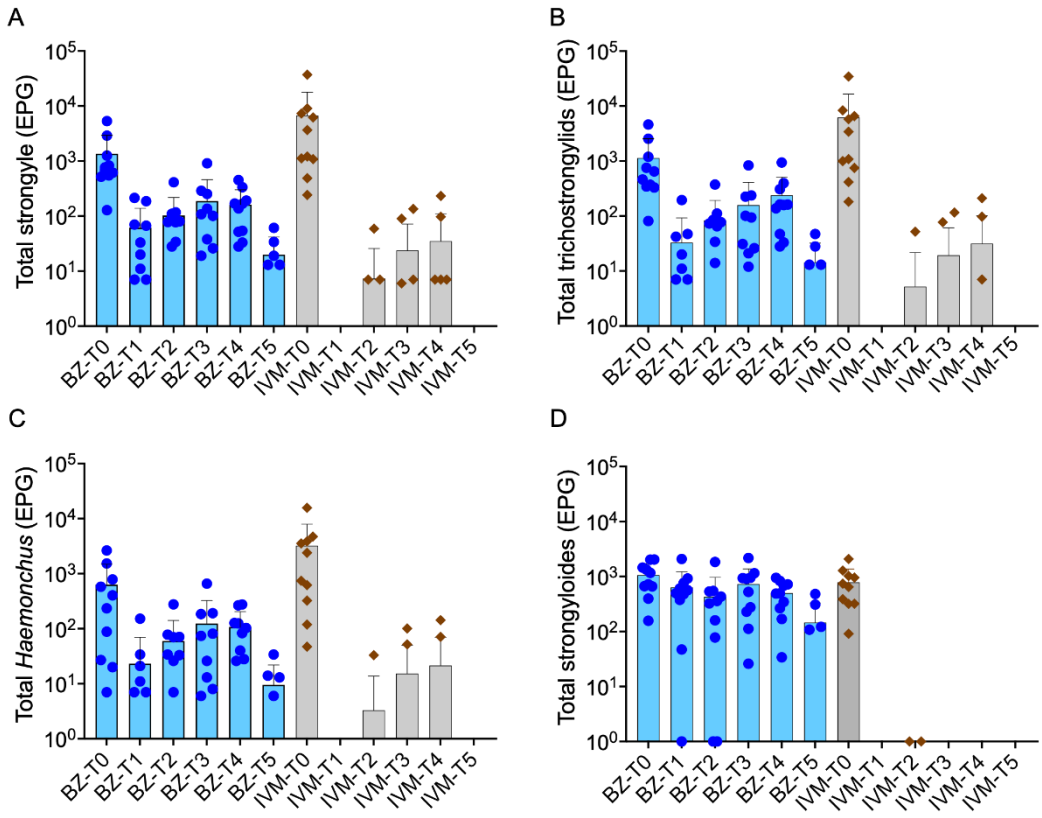

Figure 5. Faecal egg counts of (A) strongyles, (B) trichostrongyles, (C)Hc, and (D) Strongyloides sp. in FBZ- and IVM-treated animals across sampling time points T0–T5. T0 represents the pre-treatment baseline, while T1–T5 represent sequential post-treatment sampling periods. Bars indicate mean egg counts and dots represent individual animal measurements. Egg counts declined over time in both treatment groups; however, reductions were greater and more sustained in IVM-treated animals, with counts approaching zero at several post-treatment time points. In contrast, FBZ-treated animals showed more persistent egg shedding throughout the study period.

6.2 % *Haemonchus contortus* OvacyteTM vs Droplet Digital Polymerase Chain Reaction

The proportion of *H. contortus* eggs were measured across treatment groups using both OC and ddPCR (Figure 6). Within each method, differences between treatment groups (blue and brown) were observed at the evaluated time points.

Overall, a strong positive correlation was found between the percentage of *H. contortus* estimated by OC and detected by ddPCR throughout the study (Table 3). Spearman's rank correlation coefficient was 0.77 (95% CI: 0.68–0.84), indicating good agreement between the two methods. This correlation was highly significant ($p < 0.0001$; $n = 105$).

In the ddPCR data, both treatment groups showed relatively consistent mean % Hc values, with individual measurements generally clustering closely around the mean. Although some overlap between treatments was observed, the groups remained reasonably distinguishable at most time points.

Similarly, OC measurements demonstrated differences between treatment groups; however, individual values showed a wider spread, and a greater degree of overlap between treatments. This increased dispersion made distinctions between treatment groups less visually pronounced compared to ddPCR.

Despite these differences in variability and overlap, both methods showed broadly comparable trends in % Hc across treatments.

It should be noted that ddPCR data for time point 3 were not available and were therefore excluded from analysis for this method (NT).

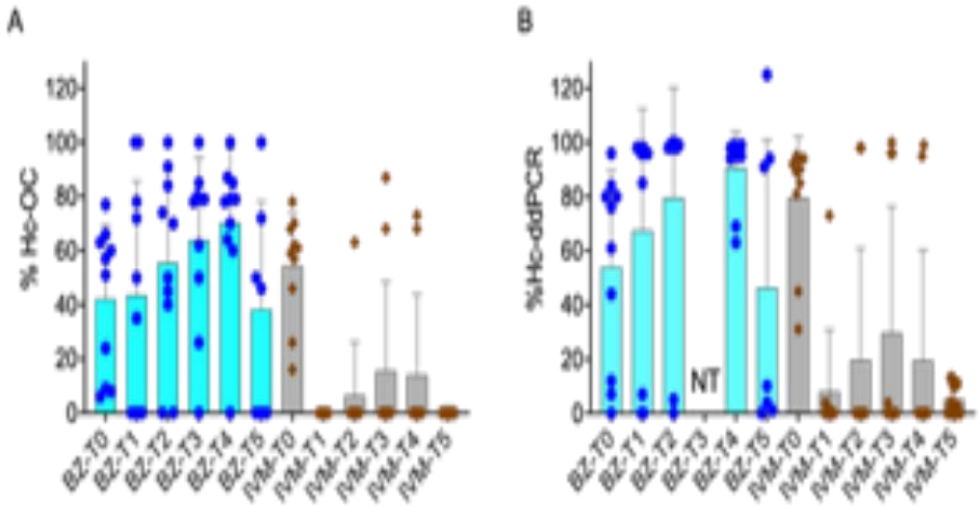

Figure 6. *Percentage of Haemonchus contortus (%Hc) measured in FBZ- and IVM-treated animals using* Ovacyte™ *(OC) and droplet digital PCR (ddPCR) across sampling time points T0–T5. T0 represents the pre-treatment baseline, while T1–T5 represent sequential post-treatment sampling periods. Bars show mean %Hc values and dots indicate individual animal measurements. Both methods showed similar temporal trends; however, ddPCR measurements displayed tighter clustering and clearer separation between treatment groups compared with OC. ddPCR data were unavailable for T3 (NT).*

Table 2. Positive\Negative status for Hc as follows

Hc	ddPCR positive	ddPCR negative
OC positive	50	9
OC negative	35	21

6.3 FECRT

The FECRT revealed marked differences in anthelmintic efficacy between FBZ and IVM across all parasite groups and sampling time points. IVM consistently showed high efficacy, with of 99–100% and narrow confidence intervals at all time points (T1–T4) for all parasites indicating full effectiveness and no evidence of resistance.

In contrast, FBZ showed reduced and variable efficacy, with clear indications of resistance in all parasite groups. For the overall strongyle population, FBZ efficacy was borderline at T1 (98%; 95% CI: 95–99), declined to 90% (81–95) at T2, and further to 83–85% (70–93) at T3–T4, indicating resistance from T2 onwards. A similar trend was observed for trichostrongyles, where efficacy decreased from borderline at T1 (97%; 90–99) to resistant at T2 (93%; 85–97), strong resistance at T3 (89%; 75–92), and severe resistance at T4 (79%; 68–87).

For *H. contortus*, FBZ efficacy was borderline at T1 (96%; 88–99) but dropped to resistant levels at subsequent time points, with reductions of 91% (81–96) at T2 and 80–83% (68–91) at T3–T4. The most pronounced resistance was observed in *Strongyloides* sp., where FBZ efficacy was consistently very low across all time points, ranging from 17% to 40% (95% CI: 5–55), indicating severe resistance throughout the study period.

Overall, these results demonstrate widespread and, in some cases, increasing resistance to FBZ across parasite taxa, whereas IVM remained fully effective under the conditions of this study.

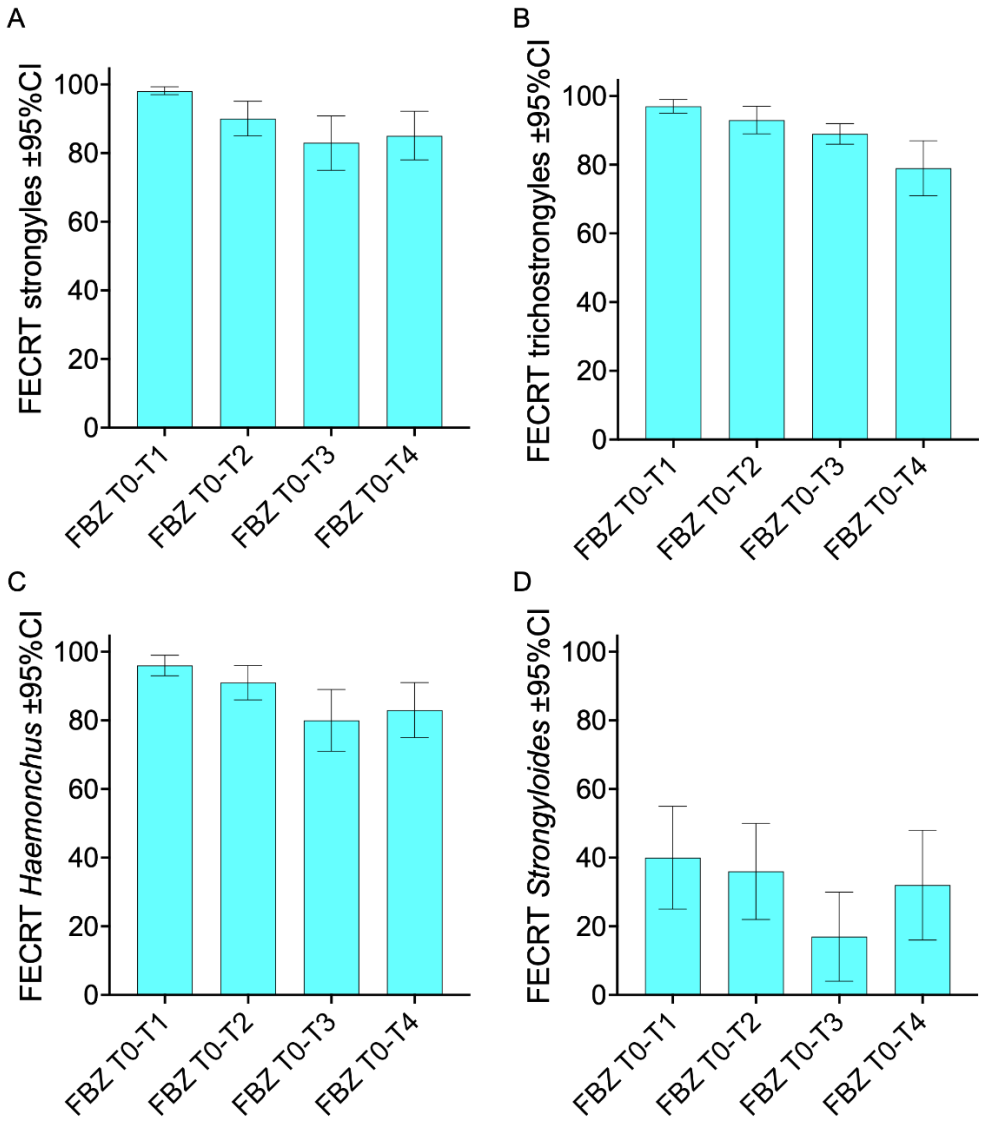

Figure 7. Faecal egg count reduction test (FECRT) results for (A) strongyles, (B) trichostrongyles, (C)Hc, and (D) Strongyloides sp. in FBZ- and IVM-treated animals across post-treatment sampling time points T1–T4. Bars represent mean percentage FECR, and error bars indicate 95% confidence intervals. IVM maintained consistently high efficacy (>99%) across all parasite groups and time points. In contrast, FBZ showed reduced and variable efficacy, with declining egg count reductions over time and evidence of resistance, particularly for trichostrongyles, H. contortus, and Strongyloides sp.

7. Discussion

7.1 Overview of the study

The present study aimed to evaluate the efficacy of IVM and FBZ on a Swedish sheep farm at several time points after treatment. In addition, it compared the performance of the AI-assisted microscopy platform with a validated ddPCR assay for the detection and quantification of the major ovine GI nematodes, particularly *H. contortus*.

In contrast to the high efficacy observed for IVM, FBZ showed a progressive loss of efficacy against several parasite groups, consistent with the increasing prevalence of benzimidazole resistance reported in Sweden and across Europe (Rose Vineer et al. 2020). Overall, OC showed good agreement with ddPCR for estimating the proportion of *H. contortus*, although ddPCR demonstrated lower variability and greater sensitivity for detecting low-level infections. These findings indicate that AI-assisted microscopy can serve as a practical tool for routine flock monitoring, while ddPCR remains the more analytically sensitive method.

7.2 Faecal egg count dynamics and anthelmintic efficacy

Following IVM administration, FECR were rapid and consistent across strongyles, trichostrongyles, *H. contortus*, and *Strongyloides* spp., with post-treatment counts approaching zero already after one week. This immediate and comprehensive clearance aligns with pharmacokinetic and field studies showing that macrocyclic lactones generally achieve highly effective clearance of susceptible GI nematodes (Six et al. 2016). This pronounced susceptible response sharply contrasts with the progressive failure of FBZ observed in this flock. Similar patterns have been documented in European and Swedish surveys, which report widespread decline in benzimidazole efficacy against GI nematodes, particularly *H. contortus*, due to long-term selection pressure.

While this pattern indicates high ML efficacy in the studied flock, it should not be generalised to all Swedish or European sheep farms. Nationwide surveys by Höglund et al. (2015) identified IVM treatment failures in several Swedish sheep flocks associated with resistant *H. contortus* strains. Furthermore, broader epidemiological reviews across Europe have demonstrated increasing ML resistance, with Rose Vineer et al. (2020) reporting substantial prevalence of resistance across European small-ruminant holdings. Consequently, the elevated IVM efficacy observed here should be interpreted as flock-specific evidence of susceptibility at the time of sampling, rather than as evidence that resistance is absent regionally.

In contrast, FBZ exhibited a marked and progressive loss of efficacy, particularly after two weeks post-treatment time point (T2) and onwards. This therapeutic decline was especially apparent in trichostrongyles (including *H. contortus*) and *Strongyloides* spp., consistent with field reports of multi-genus benzimidazole resistance (Babják et al. 2024). The progressive reduction in efficacy likely reflects the survival and continued egg shedding of resistant parasite subpopulations following treatment (Emery-Corbin et al. 2021). Such patterns are characteristic of established BZ resistance and aligns with previous observations that resistant worms become increasingly dominant once susceptible populations are removed. These findings further support the broader literature identifying BZ resistance as one of the most widespread forms of AR in small-ruminant nematodes (Kaplan & Vidyashankar 2012).

From a management perspective, these findings suggest that FBZ should not be used in this flock without continued efficacy monitoring and diagnostic follow-up. Instead, treatment decisions should ideally form part of an evidence-based parasite-control strategy that preserves refugia and minimizes unnecessary whole-flock treatment (Charlier et al. 2009).

The statistical reliability of the FECRT interpretation was enhanced by the paired pre- and post-treatment study design. This approach is recommended as it directly accounts for substantial between-animal variation in baseline egg shedding (Nielsen et al. 2014). Updated WAAVP guidelines continue to recognize FECRT

as the principal field method for evaluating anthelmintic efficacy and diagnosing resistance, while emphasizing the importance of uncertainty intervals and confidence-based interpretation (Burden et al. 2024). In the present study, the narrow confidence intervals associated with IVM supported a robust conclusion of high efficacy, whereas the lower reductions and broader uncertainty intervals for FBZ were compatible with resistance or strongly suspected resistance depending on parasite group and sampling point (Cao et al. 2023). Importantly, parasite-group-specific interpretation proved valuable because mixed infections may conceal resistant taxa if only total strongyle egg counts are considered (Panic et al. 2015). However, this study shows that *H. contortus* in particular survived the treatment with BZ.

7.3 Comparison between Ovacyte™ and ddPCR

The comparison between OC and ddPCR showed a strong positive correlation for %Hc, indicating that AI-assisted microscopy captured biologically meaningful differences in *H. contortus* abundance. This supports recent validation studies demonstrating that OC Ovine Plus/Speciation model can reliably estimate the proportion of *H. contortus* eggs within mixed strongyle populations (Ljungström et al. 2018). Similar findings were reported by Grandi et al. (2025), who showed that OC generated quantitative information comparable to the McMaster method when sample preparation and reading protocols were standardised. Likewise, Elghryani et al. (2025) observed a strong relationship between OC Ovine Plus/Speciation and peanut agglutinin staining for *H. contortus* identification. The present study extends these observations by directly comparing OC with ddPCR in naturally infected Swedish lambs across multiple treatment time points.

Despite the strong agreement between methods, ddPCR showed lower variability and tighter clustering of measurements compared with OC. This difference likely reflects the fundamental distinction between molecular and image-based diagnostics. OC relies on faecal flotation, egg recovery, image quality, and algorithm-based classification, all of which may introduce analytical variation

(Litjens et al. 2017). In contrast, ddPCR directly quantifies target parasite DNA using thousands of partitioned reactions and Poisson statistics, reducing dependence on visual egg recognition and morphological consistency (Hindson et al. 2011; Baltrušis & Höglund 2023). –Similar advantages of ddPCR have previously been demonstrated in veterinary parasitology studies targeting genus-specific ITS2 markers (Franco-Martínez et al. 2019). Consequently, the lower technical dispersion observed in the present study is consistent with earlier reports showing that molecular methods provide high analytical sensitivity and species-level specificity (Höglund et al. 2019).

The positive/negative comparison further illustrated these differences in sensitivity. Some samples were negative by OC but positive by ddPCR, suggesting that ddPCR detected low-level infections missed by AI-assisted microscopy. This may reflect low egg abundance, uneven egg distribution, damaged eggs, or the persistence of detectable DNA despite limited intact egg presence (Hindson et al. 2011). Similar improvements in diagnostic sensitivity have been described in studies comparing PCR-based methods with microscopy or larval culture (Roeber et al. 2012; Höglund et al. 2019). However, the opposite pattern was also observed, with some samples positive by OC but negative by ddPCR. Possible explanations include stochastic variation near the detection limit, differences in subsampling, DNA extraction inefficiency, PCR inhibition, or false-positive image classification caused by faecal debris resembling parasite eggs (Grandi et al. 2025). Therefore, disagreement between methods should not necessarily be interpreted as methodological failure but rather as evidence that microscopy-based and molecular diagnostics detect overlapping, though not identical, biological signals (Roeber et al. 2013).

The DNA extraction process itself likely can contribute to some variation in ddPCR performance (Roeber et al. 2013; Baltrušis et al. 2022). Previous studies evaluating extraction methods for *H. contortus* ITS2 detection found that the NucleoSpin DNA Stool kit yielded high DNA recovery and relatively low variability (Högberg et al. 2022). This is relevant because the present study also used the NucleoSpin kit after sediment recovery from OC cassettes. However, the

need for protocol optimisation demonstrates that flotation-derived sediment differs from fresh faecal material in inhibitor concentration, particle composition, and egg concentration (Han et al. 2013). Future studies should therefore include inhibition controls, extraction controls, and standardised sediment input to better distinguish and reduce biological-related from extraction-related variability.

7.4 Practical implications for parasite monitoring

A major practical advantage of OC is its speed and potential suitability for field application (Elghryani et al. 2025; Grandi et al. 2025). Although ddPCR provides high analytical sensitivity and species-specific detection, it requires specialised laboratory equipment, DNA extraction, trained personnel, and controlled laboratory conditions (Hindson et al. 2011). In contrast, OC can generate rapid results directly from prepared faecal samples and may therefore support routine flock monitoring and targeted selective treatment strategies (Grandi et al. 2025). On-site, rapid analysis of fresh samples may also be advantageous for parasites such as *Strongyloides* spp., whose eggs contain larvae and can therefore quickly hatch within few hours, potentially reducing detection accuracy if sample processing is delayed (Taylor et al. 2016).

This is particularly relevant because targeted selective treatment relies on identifying animals that contribute disproportionately to pasture contamination while preserving parasite refugia to reduce resistance selection (Kenyon et al. 2009; Charlier et al. 2014). In this context, OC may not need to match ddPCR sensitivity at extremely low infection intensities; rather, its value lies in its ability to identify clinically and epidemiologically relevant egg shedding under practical farm conditions (Lopes-Torres et al. 2020).

The present findings also highlight the value of integrating diagnostic approaches rather than viewing them as competing technologies. OC may provide rapid, on-site, scalable, and standardised flock-level monitoring, whereas ddPCR may serve as a confirmatory tool for species-level identification, low-intensity

infections, or resistance investigations. This complementary diagnostic model was previously recommended by Roeber et al. (2013), who emphasised that molecular diagnostics should complement rather than completely replace conventional parasitological methods.

7.5 Clinical relevance of parasite composition

The high proportion of *H. contortus* detected in this study is clinically important because this parasite is highly pathogenic and can cause anaemia, hypoproteinaemia, and mortality in sheep (De Assis et al. 2016). In Sweden, *H. contortus* has become increasingly significant because climatic conditions during the grazing season may support transmission and animal movement can contribute to parasite introduction and persistence within flocks (Höglund et al. 2019). Consequently, the reduced efficacy of FBZ observed here may substantially increase the risk of treatment failure and clinical haemonchosis if resistant populations continue to expand (Wang et al. 2019).

Strongyloides spp. also showed particularly poor apparent response to FBZ. This finding should be interpreted with caution, as *Strongyloides* biology differs considerably from that of trichostrongylid nematodes. The parasite has alternating free-living and parasitic generations and may rapidly amplify under favourable environmental conditions (Pila et al. 2016). Nevertheless, the persistently low FECR values suggest that FBZ did not effectively suppress *Strongyloides* egg shedding in this flock (Cao et al. 2023). These findings further support the importance of parasite-group-specific interpretation rather than relying solely on total strongyle egg counts (Roeber et al. 2013). In any case, the present findings are unprecedented in Sweden. It may be speculated that at least some of these infections might have gone unnoticed when analysing samples shipped by post using the standard McMaster technique.

7.6 Limitations and future perspectives

Several limitations should be considered. First, the study was conducted in a single naturally infected flock, which limits generalisability to other Swedish

production systems. Second, the sample size was relatively small, and the loss of animals at T5 reduced the strength of long-term comparisons. Third, ddPCR data were unavailable at one time point, limiting complete longitudinal comparison between methods. Finally, FECRT estimates remain influenced by parasite aggregation, variation in egg shedding, and sampling variability. These limitations do not invalidate the findings but indicate that the conclusions should be interpreted as strong flock-level evidence rather than universal estimates.

Future studies should include larger numbers of flocks, repeated seasonal sampling, and parallel comparison of fresh faeces, OC sediment, larval culture, and nemabiome sequencing. Including molecular resistance markers such as β-tubulin polymorphisms would further help determine whether the observed FBZ failure is genetically linked to established resistance mechanisms. Cost-benefit analyses should also be considered, as successful implementation of diagnostic technologies depends not only on analytical performance but also on affordability, labour requirements, speed, and farm-level usability.

7.7 Conclusion

In conclusion, this study demonstrated that OC provides a practically useful estimate of *H. contortus* abundance and shows strong agreement with ddPCR, although ddPCR remains more sensitive and less variable, particularly at low infection levels. IVM remained highly effective in the studied flock, whereas FBZ showed reduced efficacy consistent with AR, particularly against trichostrongyles including *H. contortus* and *Strongyloides* spp. These findings support an integrated diagnostic strategy in which rapid AI-assisted microscopy is used for routine flock surveillance and treatment decision-making, while ddPCR is applied for confirmatory species-level detection and resistance investigations. This combined approach is likely to provide a more robust framework for evidence-based and sustainable parasite control in sheep production systems and a more effective integration between research and field applications (Cao et al. 2023).

8. References

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9. Acknowledgement

I would like to express my sincere gratitude to my supervisor, Giulio Grandi for his continuous guidance, encouragement, and valuable scientific support throughout this study. His constructive feedback, patience, and expertise greatly contributed to the completion of this thesis.

I would also like to extend my sincere appreciation to my co-supervisor, Johan Höglund, for his insightful discussions, valuable suggestions, and significant support with the statistical analyses, interpretation of results, and development of the analytical models used in this research. His expertise and guidance were invaluable throughout the study.

I am grateful to the Faculty of Veterinary Parasitology and the Department of Animal Biosciences (HBIO), Swedish University of Agricultural Sciences (SLU), for providing the facilities, resources, and academic environment necessary for conducting this work.

Special thanks are extended to all laboratory staff, colleagues, and fellow students for their support and helpful discussions during the study.

Finally, I would like to express my heartfelt gratitude to my family and friends for their constant encouragement, understanding, and support throughout my academic journey.

****Use of writing tools****

I would also like to acknowledge the use of digital tools that supported the preparation of this thesis. The writing assistance tool Grammarly was used to improve grammar, spelling, and clarity of the text. In addition, generative AI models were used to support language refinement, idea structuring, and general feedback on phrasing.

The use of these tools was carried out in accordance with the guidelines for the use of generative AI and digital support tools provided by the Swedish University of Agricultural Sciences (SLU). These tools were used solely to enhance the readability and presentation of the work. All scientific content, interpretations, and conclusions are my own, and I take full responsibility for the accuracy and integrity of the material presented in this thesis.

Appendix

Table 3. Recorded sediment weights of recovered faecal samples prior to DNA extraction

	T0	T1	T2	T3	T4
I1	0.490	0.223	0.511	0.261	0.237
I2	0.337	0.348	0.371	0.298	0.230
I3	0.320	0.364	0.260	0.196	0.185
I4	0.420	0.516	0.417	0.218	0.241
I5	0.388	0.613	0.235	0.357	0.271
I6	0.390	0.374	0.509	0.304	0.403
I7	0.345	0.660	0.336	0.215	0.435
I8	0.315	0.381	0.317	0.337	0.270
I9	0.370	0.365	0.660	0.301	0.239
I10	0.513	0.749	0.455	0.402	0.284
F1	0.366	0.453	0.336	0.336	0.184
F2	0.445	0.455	0.328	0.418	0.323
F3	0.300	0.367	0.304	0.225	0.221
F4	0.363	0.443	0.267	0.374	0.283
F5	0.367	0.380	0.329	0.283	0.188
F6	0.380	0.349	0.383	0.326	0.161
F7	0.325	0.367	0.280	0.321	0.156
F8	0.405	0.381	0.301	0.344	0.249
F9	0.310	0.303	0.453	0.380	0.175
F10	0.345	0.367	0.471	0.315	0.177

Table 4. Comparison of OvacyteTM (OC) and ddPCR positive/negative detection results for Haemonchus contortus treated with fenbendazole (FBZ) and ivermectin (IVM) across six sampling time points (T0–T5).

Animal ID	Treatment	T0		T1		T2		T3		T4		T5	
		OC	ddPCR	OC	ddPCR	OC	ddPCR	OC	ddPCR	OC	ddPCR	OC	ddPCR
1	FBZ	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
2	FBZ	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-	-
3	FBZ	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-	-
4	FBZ	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
5	FBZ	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
6	FBZ	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
7	FBZ	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
8	FBZ	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
9	FBZ	+ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
10	FBZ	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-	-
1	IVM	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve
2	IVM	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
3	IVM	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-	-
4	IVM	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
5	IVM	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
6	IVM	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-	-
7	IVM	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
8	IVM	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
9	IVM	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
10	IVM	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve

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