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**The effects of a novel vaccine candidate against *Haemonchus contortus* on
the worm burden of infected sheep**

Diploma Thesis

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Submitted by

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Wien den 27.08.2025

Judith Hauth

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ZUSAMMENFASSUNG

Haemonchus contortus zählt zu den pathogensten gastrointestinalen Nematoden der kleinen Wiederkäuer. Seine hämatophage Lebensweise im Labmagen führt zu Anämien, Ödemen, Leistungsverlusten und bei schweren Erkrankungen zu Todesfällen. Haemonchosen verursachen weltweit erhebliche wirtschaftliche Verluste in der Landwirtschaft. Zunehmende Resistenzen gegenüber Anthelminthika und die begrenzte Verfügbarkeit eines wirksamen Impfstoffs unterstreichen die Notwendigkeit an neuen, alternativen Kontrollstrategien gegen *H. contortus*, zu forschen. Das Ziel dieser Studie war es, die Wirksamkeit eines neuartigen rekombinanten Impfstoffkandidaten gegen *H. contortus* hinsichtlich seiner Auswirkung auf die Wurmbürde im Labmagen infizierter Schafe zu evaluieren. Insgesamt wurden 35 männliche Schaflämmer in fünf Gruppen eingeteilt, darunter eine negative Kontrollgruppe, eine positive Kontrollgruppe, eine Gruppe, welche den kommerziellen Impfstoff erhielt (Barbervax®), sowie zwei Gruppen die entweder einen glykoengineerten Antigen-Cocktail (GErH11) oder einen nicht-glykoengineerten Antigen-Cocktail (nGErH11) erhielten. Auf das definierte Impf- (drei Impfdosen, s.c.) und Infektionsprotokoll (eine Dosis à 5,000 L3-Larven), folgte die Bestimmung der Wurmbürde. Der Barbervax® Impfstoff reduzierte die Wurmbürde um 86,36 % ($p < 0,01$), die GErH11- bzw. nicht-GErH11-Impfstoffkandidaten erreichten eine Reduktion um 25,36 % bzw. 13,72 %, wobei beide Ergebnisse nicht statistisch signifikant waren. Darüber hinaus reduzierte Barbervax® das Verhältnis der weiblichen zu den männlichen Würmern von 1,29 (pos. Kontrolle) auf 0,59, GErH11 und nGErH11 hatten keinen signifikanten Einfluss auf das Verhältnis der weiblichen zu den männlichen Würmern. Dieses Verhältnis spiegelt einen wichtigen Kontrollparameter in Bezug auf die Unterbrechung des Pathomechanismus wider. Die Ergebnisse deuten darauf hin, dass die getesteten rekombinanten Impfstoffkandidaten zwar einen gewissen Schutz gegen *H. contortus* aufbauen, derzeit jedoch nicht an die Wirksamkeit des getesteten kommerziell verfügbaren Impfstoffes (Barbervax®) heranreichen. Weitere Forschung zur Optimierung des Antigendesigns, ist erforderlich um die Immunantwort der Vakzine sowie die erfolgreiche Anwendung in der Praxis zu verbessern.

ABSTRACT

Haemonchus contortus is a highly pathogenic gastrointestinal nematode affecting small ruminants. Its Pathomechanism includes blood-feeding in the abomasum, leading to anaemia, oedema, loss of productivity and in severe cases even death of the animals, resulting in serious economic consequences for the livestock industry worldwide. Due to an extensive anthelmintic resistance and the restricted availability of vaccines, new approaches to control *H. contortus* are required. This research project aimed to evaluate the efficacy of two new recombinant vaccine candidates against *H. contortus* in sheep, focusing on their impact on worm burden in the abomasum of infected sheep. A total of 35 male lambs were divided into five groups, including negative and positive controls, one group receiving a commercial vaccine (Barbervax®) two groups receiving either a glyco-engineered antigen cocktail (GErH11) or a non-glyco-engineered antigen cocktail (nGErH11). Following a standardised vaccination (three doses, administered s.c.) and challenge protocol using one dose of 5,000 infective L3 larvae administered orally per sheep, worm counts were assessed *post-mortem*. Barbervax® resulted in a significant 86.36 % reduction in worm burden ($p < 0.01$), while GErH11 and non-GErH11 vaccines decreased the worm burden in the abomasum by 25.36 % and 13.72 %, however both results were not statistically significant. While Barbervax® reduced the ratio of female-to-male worms from 1.29 (pos. control) to 0.59, the recombinant vaccines had no significant effect on the ratio of female-to-male worms, which is a valuable measure of parasite transmission. The study concludes that although there was partial efficacy against *H. contortus* demonstrated by the two tested recombinant vaccine candidates (GErH11 and nGErH11), they were unable to match the efficacy of the commercial vaccine (Barbervax®). Therefore, further optimisation of antigen design is needed to enhance immunogenicity and field efficacy.

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LIST OF ABBREVIATIONS

ANOVA Analysis of variance

ARRIVE Animal Research: Reporting of In Vivo Experiments

BCS Body condition score

BW Bodyweight

e.g. for example

E/S antigen excretory/secretory antigen

FAO Food and Agriculture Organisation of the United Nations

FAMACHA[©] FAffa MAlan CHArt

GErH11 glyco-engineered antigen cocktail

L1 first stage larvae

L2 second stage larvae

L3 third stage larvae

L4 fourth stage larvae

L5 fifth stage larvae

nGErH11 non-glyco-engineered antigen cocktail

n.a. not applicable

1. INTRODUCTION

Haemonchus contortus is recognised as one of the most threatening gastrointestinal nematodes affecting small ruminants.

The Pathomechanism of *H. contortus* infections primarily involves blood feeding in the abomasum of small ruminants, leading to anaemia, general weakness, oedema, and severe infections that often result in the death of goats and sheep, causing significant economic losses to the livestock sector (1). According to data from 2020 provided by the Food and Agriculture Organisation, sheep and goats constitute a significant portion of the global livestock population, with numbers reaching 1.26 billion sheep and 1.13 billion goats (2). As a result of the widespread use of anthelmintic medications, *H. contortus* has developed a wide-ranging resistance to anthelmintics that have been used for decades, often due to incorrect dosing or generally inadequate administration (3). The life cycle of *H. contortus*, which can include hypobiosis depending on environmental and host-related factors, makes the parasite very robust under varying situations. Additionally, the fact that adults live in the abomasum, where they ingest blood from their host, makes this parasite an interesting candidate for a potential vaccine. Being major producers of sheep worldwide, Australia and Scotland were especially interested in creating a vaccine candidate and started looking into possible vaccines in the 1990s (4). The Barbervax® vaccine was developed at the Moredun Institute in Scotland and was brought to market in 2014 through a collaboration with the Albany Laboratory of the Department of Agriculture and Food in Western Australia.

Barbervax® uses native antigens, such as H11, which are taken from sheep previously infected with *H. contortus* and subsequently killed. H11, a so-called "hidden antigen," has been of interest for researchers as it has shown to play a significant role in the infection process and is able to generate robust protective immunity (5). Alongside H11, the somatic antigen rHc23 has also been studied, with recombinant forms of rHc23 tested for their efficacy in protecting lambs against *H. contortus* infections (6).

The approach of using native H11 has demonstrated high efficacy, achieving protective levels between 75 % and 93 % in both worm burden and faecal egg count (7).

As the production of the described vaccine is limited to the availability of slaughtered sheep and their abomasa, the total process is very expensive, highly dependent and from an ethical point of view questionable.

The severe anthelmintic resistance and the fact that there is currently only one vaccine available on the market, which is only available in Australia, South Africa, have made the need for a new, widely accessible, and effective vaccine candidate increasingly demanding in recent years (8). This study aims to evaluate the protective effect of a novel vaccine candidate against *H. contortus*. Specifically, the study compares the effectiveness of a glycoengineered vaccine candidate with that of a non-glycoengineered vaccine candidate, focusing on their effect on the worm burden found in the abomasum of infected sheep. It is hypothesised that “the novel vaccine candidate will significantly reduce the *Haemonchus contortus* worm burden in the abomasum of infected sheep”.

2. LITERATURE REVIEW

2.1. *HAEMONCHUS CONTORTUS*

The genus *Haemonchus* belongs to the *Trichostrongylidae* superfamily. *Trichostrongylidae* are considered to be the most pathogenic nematodes affecting small ruminants (9). Together with species of *Teladorsagia*, members of the *Haemonchus* genus are among the primary gastrointestinal parasites of concern in ruminants. While *Haemonchus* is hematophagous (blood-feeding) and can cause severe blood loss and anaemia, *Teladorsagia* primarily damages the abomasal mucosa, leading to reduced nutrient absorption and impaired growth. This allows them to cause significant physiological damage across a range of ruminant hosts (10, 11). The *Haemonchus* genus includes at least 12 species, the most predominant being *Haemonchus placei* and *Haemonchus contortus*. While *H. placei* is typically associated with larger ruminants such as cattle, *H. contortus* primarily parasitises small grazing ruminants, including sheep and goats. This species has a broad geographical distribution and colonises the abomasum of both domesticated and wild ruminants, where it feeds on blood and contributes to significant economic and health-related impacts (12).

Infections with trichostrongylids in ruminants primarily occur during the grazing season. The epidemiological pattern of the infection is seasonal, being mainly influenced by temperature and moisture, which are critical factors for the development and survival of free-living larval stages. Generally, this process follows a seasonal, cyclic pattern influenced by climatic conditions, geographical location, and the elevation of pastures (13).

To identify the periods when the risk of infection is highest, it is crucial to understand the seasonal patterns. This knowledge forms the basis for strategic control programmes, which aim to minimise parasite burdens through effective anthelmintic treatments and appropriate pasture management interventions (13).

As shown in **Figure 1**, the life cycle of *H. contortus* is direct and does not require an intermediate host, consisting of both free-living and parasitic stages (12). Under suitable environmental conditions, warm temperatures and high humidity, eggs can hatch in the faeces within 1–2 days into first-stage larvae (L1), which feed on bacteria and moult into second-stage larvae (L2). These then develop into the infective third-stage larvae (L3), which retain the L2 cuticle as a protective sheath and do not feed.

The L3 larvae migrate from faeces onto surrounding vegetation, where they are ingested by grazing animals (1, 12). Following ingestion, L3 larvae exsheath in the rumen and migrate to the abomasum, where they penetrate the gastric glands and develop into fourth-stage larvae (L4) and fifth-stage larvae (L5), eventually maturing into blood-feeding adult worms. The prepatent period, defined as the interval between infection to the first excretion of eggs in the faeces of the host, is typically 18–21 days (1, 12).

Clinical signs of haemonchosis are primarily associated with anaemia, resulting from the blood-feeding activity of *H. contortus*. This begins with the fourth larval stage (L4) and continues with adult worms residing in the abomasum. Clinical signs of anaemia usually become evident 10 to 12 days post-infection, depending on factors as the immune status of the host, host age, the intensity of the infection as well as the form of infection. Each adult worm consumes an estimated amount of 30 to 50 μL of blood per day. A sheep harbouring 1,000 worms could consequently experience a daily blood loss of up to 50 mL (14). Another key pathological concern of *H. contortus* infection is hypoproteinaemia, particularly hypoalbuminemia, resulting from the parasite's hematophagous activity.

The subsequent reduction in oncotic pressure promotes fluid leakage into the interstitial spaces, leading to oedema. A clinical sign in small ruminants is the so-called “bottle jaw”, characterised by soft, pitting oedema in the intermandibular region (1). Infected animals may also exhibit general weakness, progressive weight loss, and reduced productivity in terms of wool, milk, or meat yield (9). As shown in **Figure 2**, *H. contortus* individuals are easily recognisable on the abomasal mucosa. Typically, male worms measure 1.00–2.00 cm in length, while female individuals are larger and measure 1.80–3.00 cm in length. Female worms display a characteristic “barber’s pole” appearance, which results from the red intestinal tract, filled with host-derived haemoglobin spiralling around the white uterus (13).

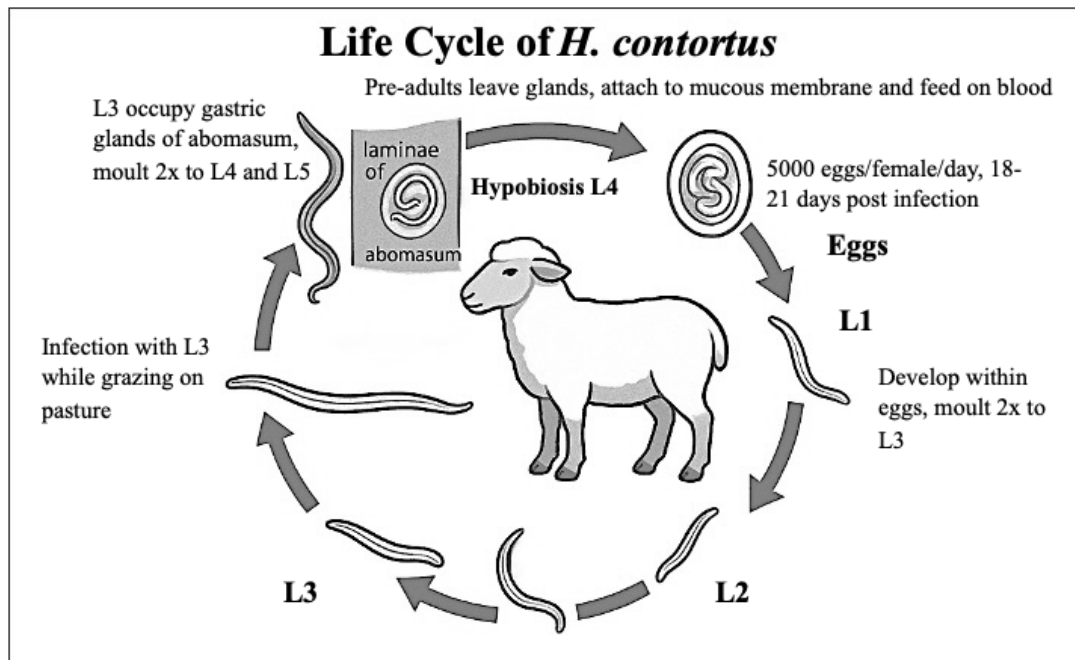


Figure 1 Life cycle of *H. contortus* showing the different parasitic development stages as well as its Pathomechanism, Modified from Adduci et al. (15)

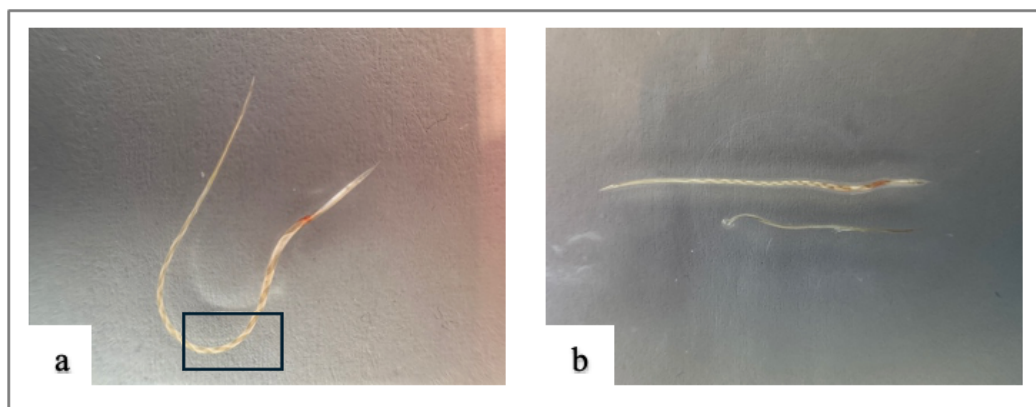


Figure 2 *Haemonchus contortus* **a** female *H. contortus* **b** female and male *H. contortus* in comparison © Floriana Sajovitz-Grohmann/Vetmeduni Vienna (16)

2.2. *H. CONTORTUS* CONTROL STRATEGIES AND VACCINE RESEARCH

Haemonchosis remains a threat to the global viability of small ruminant farming creating severe health challenges and substantial financial burdens, which stimulate extensive research into its epidemiology and control strategies (15). The parasite's ability to adapt rapidly to environmental changes, especially to climate warming as well as to develop resistance against all major classes of anthelmintics (benzimidazoles, imidazothiazoles, and macrocyclic lactones), poses a major challenge to sustainable parasite control (17, 18).

In response to growing drug resistance, research has shifted toward integrated parasite management strategies. These include selective breeding for host genetic resistance, nutritional supplementation to improve resilience, pasture management and the development of vaccines. Breeding programs have shown that certain sheep and goat breeds possess heritable resistance traits, and genomic selection is increasingly being explored as a tool to accelerate the integration of such qualities into flocks (19). Meanwhile, nutritional interventions, particularly protein-rich diets and plant-based compounds such as condensed tannins, have demonstrated potential in reducing worm burdens and improving host resilience (20). Among all control strategies, the development of a protective vaccine is regarded as one of the most promising and sustainable long-term solutions. Especially as there is currently only one commercial vaccine available, Barbervax[®], which is based on native gut membrane antigens (particularly H11 and H-gal-GP) derived from adult *H. contortus* worms (21, 22, 23, 24). Barbervax[®] has been shown to significantly reduce worm burdens and faecal egg output in vaccinated animals and has been licensed for use in sheep in Australia, and parts of South Africa. However, its use is limited geographically, the vaccine requires multiple booster injections, and is based on native antigen extraction, which poses scalability and cost-efficiency challenges (12). To address these limitations, research has increasingly focused on recombinant antigen vaccines. Recombinant versions of H11 and other gut antigens, including rHc23, have been developed with the goal of producing consistent and scalable vaccines (6, 25). Recombinant vaccines differ in antigen type: H11 is a “hidden” gut membrane antigen, normally inaccessible to the host immune system during infection (26, 27), whereas rHc23 is an somatic antigen that is actively released and directly encountered by the host (6, 29). These differences influence immunogenicity and the observed protective efficacy (12). Recombinant vaccines have generally elicited lower protection than native antigens, with high individual variability in efficacy (29, 30, 31). Efforts

to optimise recombinant vaccines include improving antigen expression systems, evaluating adjuvant formulations, and designing multi-epitope vaccines through immunoinformatics and reverse vaccinology (25, 32). Female *H. contortus* are key targets for vaccination due to their high fecundity (5,000–10,000 eggs per day) and significant blood consumption (~0.05 mL/day), which contribute substantially to pathogenicity (33). Native gut antigen vaccines, such as H-gal-GP and H11, have been shown to impair female worm development and survival, reducing egg shedding (21, 27). Recombinant formulations, however, have not yet consistently matched this effect, potentially due to differences in glycosylation, epitope conformation, or antigen presentation (30). Ongoing research continues to explore strategies to enhance the effectiveness of recombinant vaccines, including optimising antigen dose, evaluating adjuvants, and conducting trials in diverse sheep breeds and under field conditions to ensure broad applicability (6, 12).

3. MATERIAL AND METHODS

3.1. ANIMAL EXPERIMENT

ETHICS

The study (34) was approved by the Ethics and Animal Welfare Committee of the University of

Veterinary Medicine, Vienna in accordance with the University's guidelines for Good Scientific Practice and authorized by the Austrian Federal Ministry of Education, Science and Research (vaccine trial: GZ:2023-0.734.950; *H. contortus* L3 production: GZ: 2022-0.599.404) in accordance with current legislation. The study was conducted according to the **ARRIVE Guidelines** (35).

The vaccine trial was carried out from January to May 2024, with scheduled dates for the three vaccinations, sample collections of blood, saliva and faeces, clinical examinations, infection with L3 larvae, and at the final stage of the trial, the euthanasia of the sheep and the following worm count in the abomasum.

Thirty-five Lacaune x Jura male lambs from a Lower Austrian dairy sheep farm were selected for the vaccination trial, they were housed indoors and never went onto pasture. To assess their parasitological status, faecal samples were collected and examined in September 2023 for gastrointestinal nematodes, with negative results. In December 2023 they received oral anthelmintic treatment. The treatment consisted of 0.2 mg/kg bodyweight (BW) of ivermectin (Ivomec® 10 mg/ml, Boehringer Ingelheim Animal Health) subcutaneously. On the first day of the experiment (January 2nd and 3rd, 2024), the sheep received an oral treatment consisting of 5 mg/kg BW fenbendazole (Panacur® 250 mg, Intervet GesmbH, Vienna), 7.5 mg/kg BW netobimin (Hapadex® 50 mg/kg, Intervet GesmbH, Vienna) and 20 mg/kg BW toltrazuril (Baycox® Multi 50 mg/ml, Bayer Animal Health GmbH, Leverkusen, Germany).

On January 02, 2024, at the average age of four months, the 35 lambs were transported and moved into an indoor barn (**Figure 3**) dedicated to animal studies at the campus of the University of Veterinary Medicine in Vienna.

After they arrived at the clinic, a clinical examination was performed, faecal samples, as well as saliva samples, were taken, and blood samples were taken from the jugular vein using a vacutainer system.

The 35 lambs were then weighed and randomised to allocate them to 5 groups, resulting in 5 groups of 7 individuals.

Each of the groups was moved in a box, equipped with straw as litter, hay and water *ad libitum*.

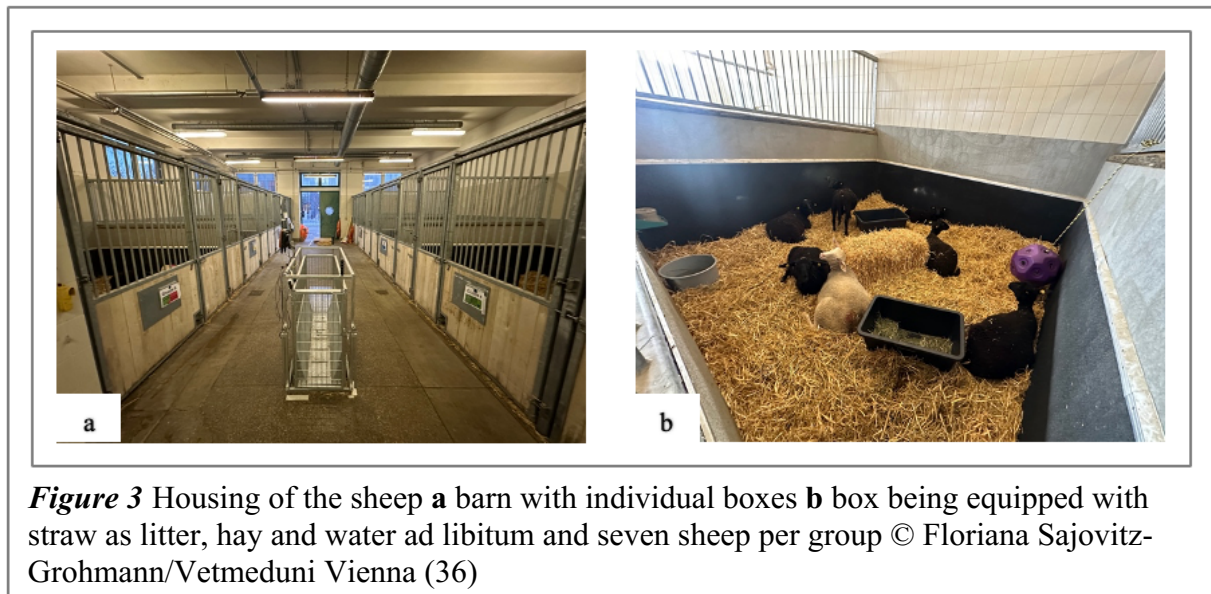


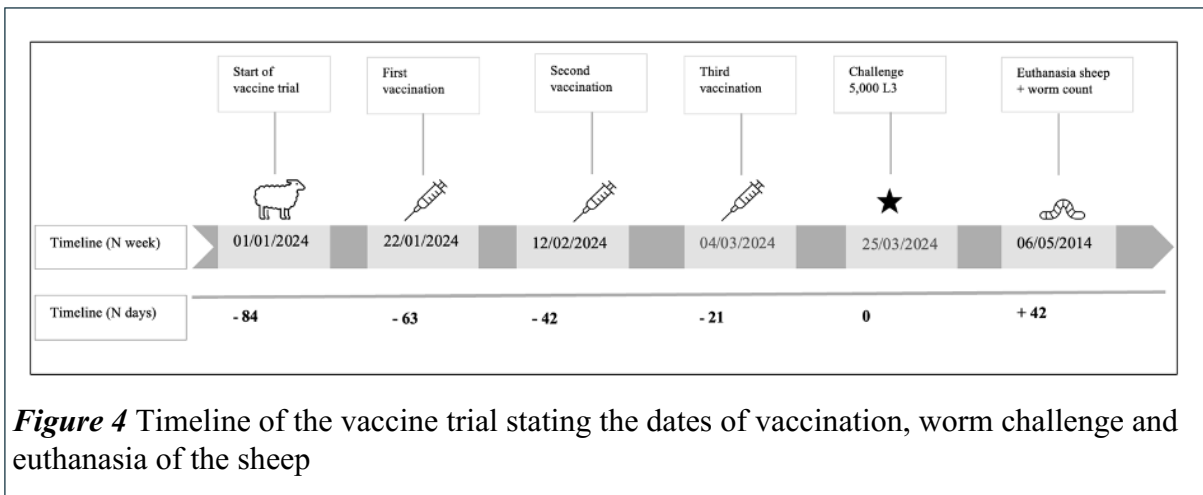
Table 1 Definition of the groups within the vaccine trial, stating Challenge, Vaccination and Abbreviation of each group

Group	Challenge	Vaccination	Abbreviation
Negative control	No	No	Negative
Positive Control	Yes	No	Positive
Barbervax [®]	Yes	Yes	Barbervax [®]
Glycoengineered recombinant antigen cocktail	Yes	Yes	GErH11
Non-glycoengineered recombinant antigen cocktail	Yes	Yes	nGErH11

3.2. TRIAL TIMELINE

As shown in **Figure 4**, the trial started on January 02, 2024, over the 19 week- trial period clinical assessments, differential blood counts, serum antibody responses as well as faecal egg counts were assessed at least once per week. The trial included three vaccination dates: January 24, 2024; February 14, 2024; and March 6, 2024. Sheep in Group 3 were vaccinated with Barbervax® (Wormvax Australia Pty Ltd., Albany, WA, Australia; imported from UK via Merlin Vet, Kelso, UK). Two novel vaccine candidates were evaluated: sheep in Group 4 were administered the GERH11 antigen cocktail (GERH11), whereas sheep in Group 5 received the non-GERH11 antigen cocktail (nGERH11). Sheep were vaccinated with 1 ml of the vaccine subcutaneously.

On March 27, 2024, sheep belonging to Groups 2 through 5 were exposed to a challenge involving a dose of 5,000 L3 larvae (isolate from Boehringer Ingelheim Vetmedica GmbH, Kathrinenhof Research Center, Rohrdorf, Bavaria, Germany) per individual sheep, which was administered orally using a 2 ml disposable plastic pipette (dosage 2 ml).



After the infection, the trial continued for 40 days, with clinical assessments, blood samples, faecal samples twice a week, and saliva samples once a week, which were analysed in the Central Laboratory of the University of Veterinary Medicine Vienna, as well as from the Team of the research group. The blood samples which included differential blood counts, Immunoglobulin concentrations, together with the evaluation of the body weight and clinical assessments of the sheep, were used to address specific research questions that are addressed by the other students involved in the trial.

3.3 CLINICAL ASSESSMENTS

Starting on the day of arrival (January, 02.01.2024) and continuing during the 5 months (until May, 06.05.2024) of the study, a standardised clinical examination was performed that included an assessment of the conjunctivae using the FAMACHA® score, the auscultation of the lungs and the rumen (using the 3M Littmann Classic III Stethoscope (3M, St. Paul, MN, USA), assessment of the body temperature with a digital thermometer (Microlife VT1931 Microlife, Widnau, Switzerland), the bodyweight (using The Gallagher W0 digital scales (Gallagher Europe, Groningen, The Netherlands), as well as the assessment of the body condition score (BCS) (**Fig.7**) and the Dag score (**Fig 8**). The clinical assessments (37) and sample collection that were performed during the trial, are displayed in **Figure 5**.



Figure 5 Clinical assessments and sample collection **a** clinical examination **b** blood sampling **c** saliva sampling © TopVetScience Team (38)

The FAMACHA[®] score relates to a system designed for treating only those sheep or goats that are severely impacted by and unable to withstand a *H. contortus* infection. Consequently, clinical anaemia serves as a determinant by examining the colour of the mucous membranes and scoring them according to the system illustrated in **Figure 6**. FAMACHA[®] is an acronym resulting from the name of the inventor of the system, Dr. Faffa Malan (FAffa MAlan CHArt) (39).

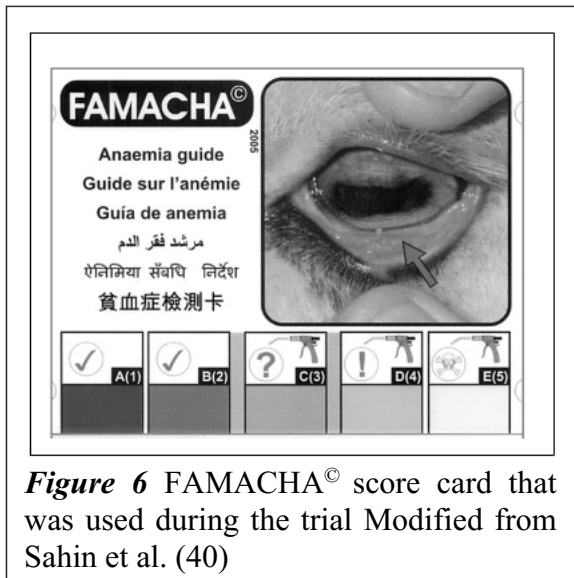
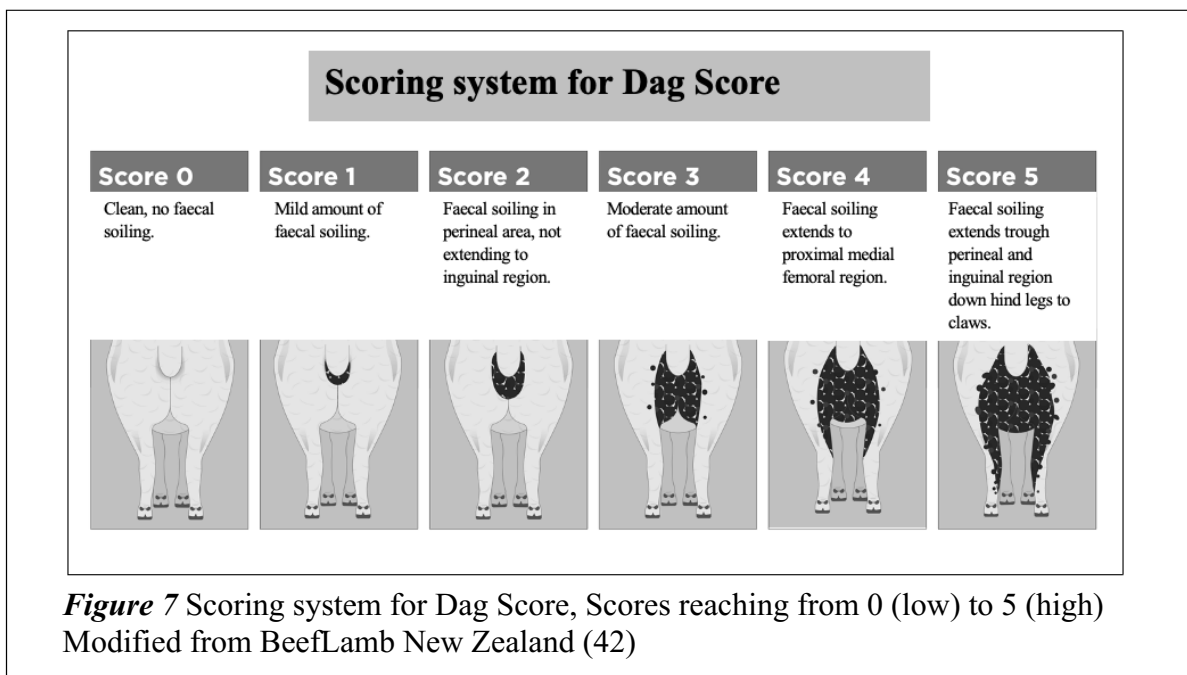


Figure 6 FAMACHA[®] score card that was used during the trial Modified from Sahin et al. (40)

The Dag scoring system is a standardised method used to evaluate the accumulation of faecal soiling, which is referred to as dags, on the perineal region and wool of sheep. This parameter is of significant relevance in both animal welfare and production efficiency, as high dag scores are associated with increased risk of flystrike (myiasis), reduced wool quality, and labour-intensive management practices (41).

As shown in **Figure 7**, dag scores typically range from 0 to 5, with higher scores indicating more severe faecal soiling. A score of 0 represents a clean breech area with no visible soiling, while a score of 5 reflects extensive accumulation of dried or moist faecal material, often resulting in large clumps of dags that can hinder normal movement and predispose the animal to parasitic infestation. The scoring is usually performed visually or through manual inspection of the breech and crutch areas (41).



Body Condition Scoring (BCS) as displayed in **Figure 8** is a practical, non-invasive method for assessing the nutritional status and energy reserves of sheep by evaluating the amount of muscle and subcutaneous fat over the lumbar region, particularly around the spinous and transverse processes of the lumbar vertebrae. Scores typically range from 1 (thin) to 5 (obese), with steps of 0.5 allowing for more accurate assessment (43).

The optimal BCS for most production stages in sheep generally lies between 2.5 and 3.5, depending on factors such as breed, physiological status, and management goals (43).

BCS is a valuable tool for guiding feeding strategies, monitoring health, and optimising reproductive performance. The assessment is conducted manually by palpating the longissimus dorsi muscle and the associated fat cover along the lumbar spine, allowing for consistent evaluation regardless of wool length or body size. Its ease of use, low cost, and strong correlation with the condition of the animal make BCS an essential component of modern flock management (43).

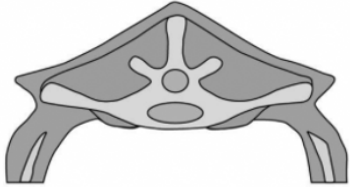
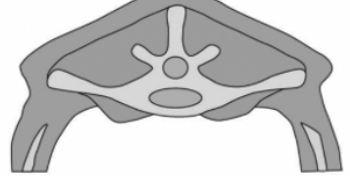
	SCORE 1 Spinous and transverse processes of the lumbar vertebrae are sharply defined and easily palpable. Minimal to no subcutaneous fat is present, and the musculature of the longissimus dorsi is markedly reduced.
	SCORE 2 Spinous processes remain palpable but exhibit slight rounding. Transverse processes can still be distinguished individually. Longissimus dorsi muscle mass is modest, with limited subcutaneous fat deposition.
	SCORE 3 Spinous and transverse processes are palpable only with firm pressure and are smoothly contoured. The longissimus dorsi muscle is well-developed, and there is a moderate and even fat cover, indicating an optimal body condition.
	SCORE 4 Spinous processes are not individually palpable and appear as a continuous line. Transverse processes are obscured by subcutaneous fat. Longissimus dorsi musculature is full and accompanied by a significant fat layer.
	SCORE 5 Spinous and transverse processes are completely obscured by thick subcutaneous fat, even under firm pressure. Longissimus dorsi musculature is markedly rounded with excessive fat accumulation.

Figure 8 Body condition score ranging from 1 (low) to 5 (high) Modified from Hook Norton Vets (44)

3.4 WORM COUNT

The final step of the vaccine trial, which involved counting the worms present in the abomasum, was completed on May 9, 2024. To gather samples for RNA analyses, the sheep (N = 35) were euthanised in groups in a humane manner, fulfilling the Austrian welfare regulations and guidelines. Upon completion of the euthanasia process, the abdomen of each sheep was opened for the collection of tissue samples from the ileum, lymph nodes, abomasum, and rumen. The samples were inserted into BiopSafe (BiopSafe®, Vedbæk, Denmark) or Eppendorf tubes (Eppendorf, Hamburg, Germany) and directly preserved in liquid nitrogen.

Each extracted abomasum was placed in a 10-liter bucket, with one bucket designated for each animal, leading to a total of 35 buckets. The buckets were immediately after the euthanasia and sample collection process finished, taken to the Pathology Institute at the Veterinary University of Vienna for counting *H. contortus*. The buckets were stored in a cooling chamber overnight.

Figure 9 displays the whole worm count process. The abomasa were opened and flushed with tap water at the institute, while the abomasal fluid was sieved (mesh width of 150 µm), with any excess water being returned to its original buckets. **10 % formalin** was added to the buckets to preserve the organ and the worms. To aid in the identification and counting of worms, each abomasum was positioned in a white plastic tray.

Worm counting was carried out over three consecutive days by thoroughly examining the abomasal mucosa using forceps and a needle, collecting each worm and putting it on a petri dish. The sex of each identified worm was determined by looking at the characteristic blood-filled gut, spiraling around the uterus of the females and the spicules and bursal flaps of the males, and documented (45, 46). Once the worm count from the abomasum was finished, counting proceeded with a careful examination of the excess abomasal fluid that was washed into the bucket. A Petri dish was repeatedly filled with the excess abomasal fluid, and the worm count was conducted by using a needle to extract each worm and transfer it to another Petri dish filled with **0.9 % NaCL**, while identifying its sex at the same time.

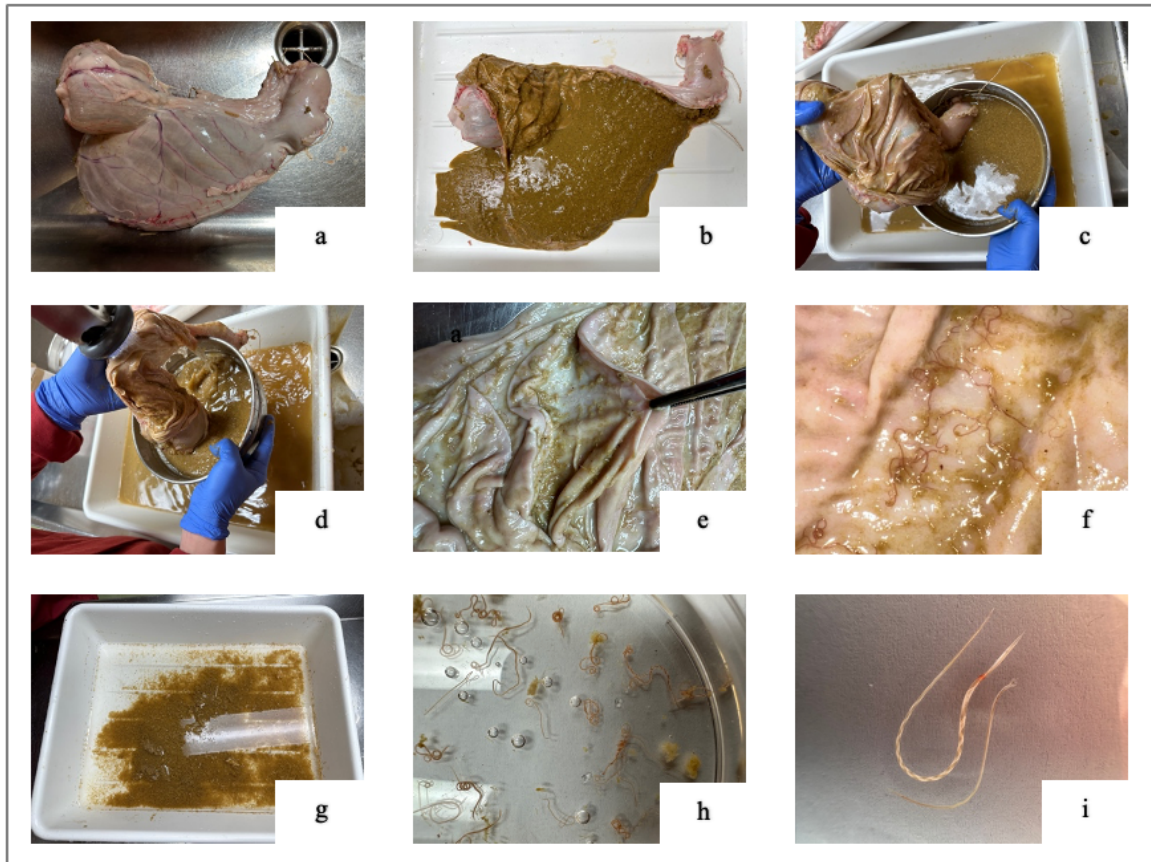


Figure 9 Worm count methods Part 1 **a** abomasum **b** opened abomasum **c** filter abomasal fluid through a sieve **d** rinse abomasum thoroughly Part 2 **e** worm count within the abomasum **f** *H. contortus* adults on abomasal mucosa **g** abomasal fluid in plastic tray **h** *H. contortus* individuals in petri dish **i** female and male *H. contortus* © Floriana Sajovitz-Grohmann/Vetmeduni Vienna (47)

3.5 STATISTICS

The statistical analysis was conducted using Microsoft Excel 2016 and IBM® SPSS® Statistics Version 29 (IBM®, New York, USA). For the number of worms per group (Group 1 to Group 5), the following values were calculated: median, range (minimum, maximum), and the 25th and 75th percentiles. An extreme outlier was defined as: >75th percentile plus 3 times the interquartile range (IQR). The number of worms per group was log-transformed. Subsequently, the Kolmogorov-Smirnov test, including the Lilliefors correction, was performed to check whether the data were normally distributed. The number of worms per group (Group 1 to Group 5) was normally distributed. A one-way analysis of variance (ANOVA) was then conducted. The Sidak test was used as a post hoc test, with the confidence interval set at 0.95. The significance level was set at $p \leq 0.05$.

4. RESULTS

4.1 NUMBER OF WORMS PER GROUP

Worm burdens of 35 experimentally infected sheep were counted to assess the abundance of *H. contortus* across groups. The boxplot in **Figure 10** shows the distribution of worm counts per group. Group 2 (positive control) had the widest range (673–3286) and the highest median (2587). Group 3 (Barbervax®) showed the lowest median (353) and the narrowest range (107–1029). Group 4 (GEnH11) displayed a wide range (1025–3332) with a median of 1931, while Group 5 (nGEnH11) exhibited a narrower range (2025–3064) with a median of 2232. The exact descriptive statistics (median, minimum, maximum, and quartiles) are provided in **Table 2**.

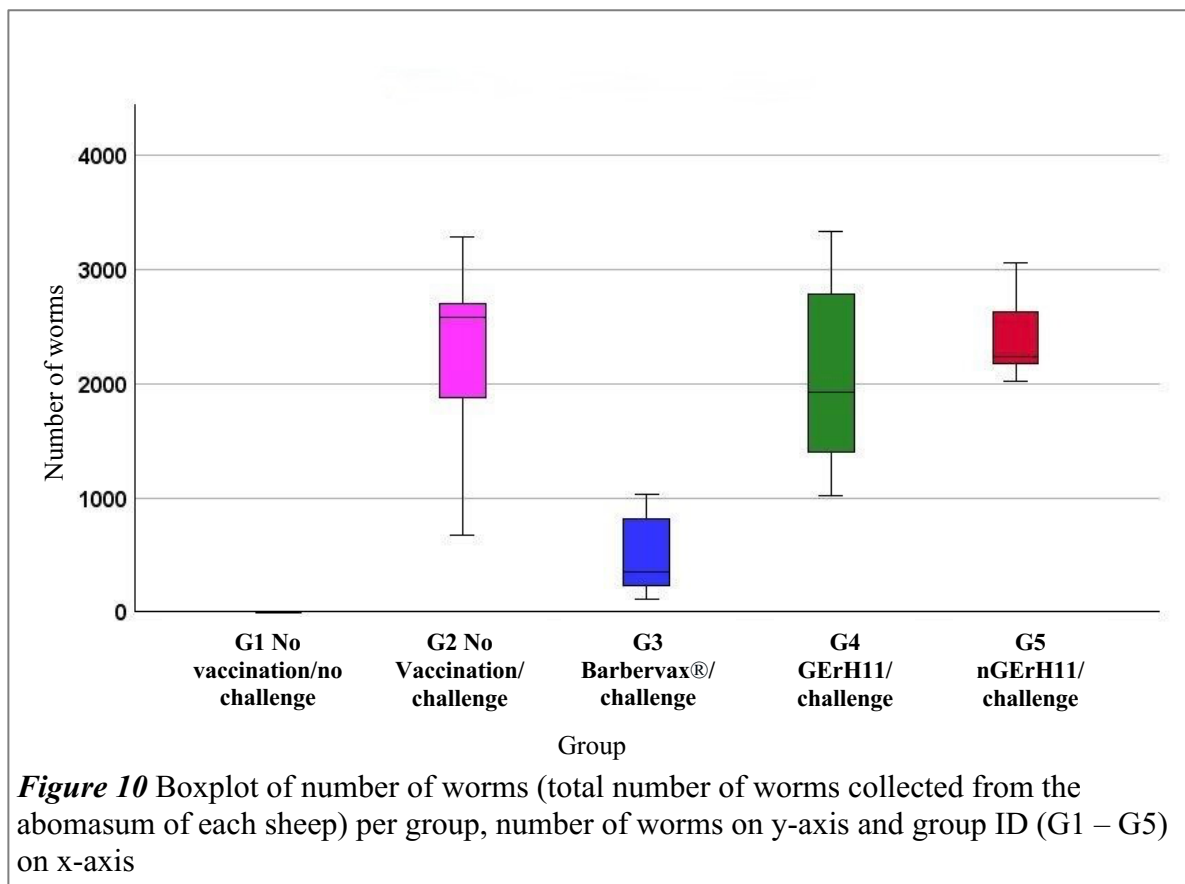


Table 2 Descriptive Statistics of number of worms by group, stating Median, Minimum, Maximum, 25th Percentile and 75th Percentile

Group	1	2	3	4	5
Median	n.a.	2587.00	353.00	1931.00	2232.00
Minimum	n.a.	673.00	107.00	1025.00	2025.00
Maximum	n.a.	3286.00	1029.00	3332.00	3064.00
25th Percentile	n.a.	1299.00	182.00	1080.00	2147.00
75th Percentile	n.a.	2784.00	920.00	3235.00	2725.00

4.2 ANOVA ANALYSIS AND POST-HOC-SIDAK TESTS

The performed ANOVA analysis showed a statistical significance between the groups ($p = 0.0001$).

Post-hoc Sidak tests revealed no statistically significant differences between Group 2 and Group 4 ($p = 0.99$) or between Group 2 and Group 5 ($p = 0.998$). However, a significant difference was observed between Group 3 and Group 4 ($p = 0.002$) as well as between Group 3 and Group 5 ($p = 0.0002$). The results are listed in Table 3 below.

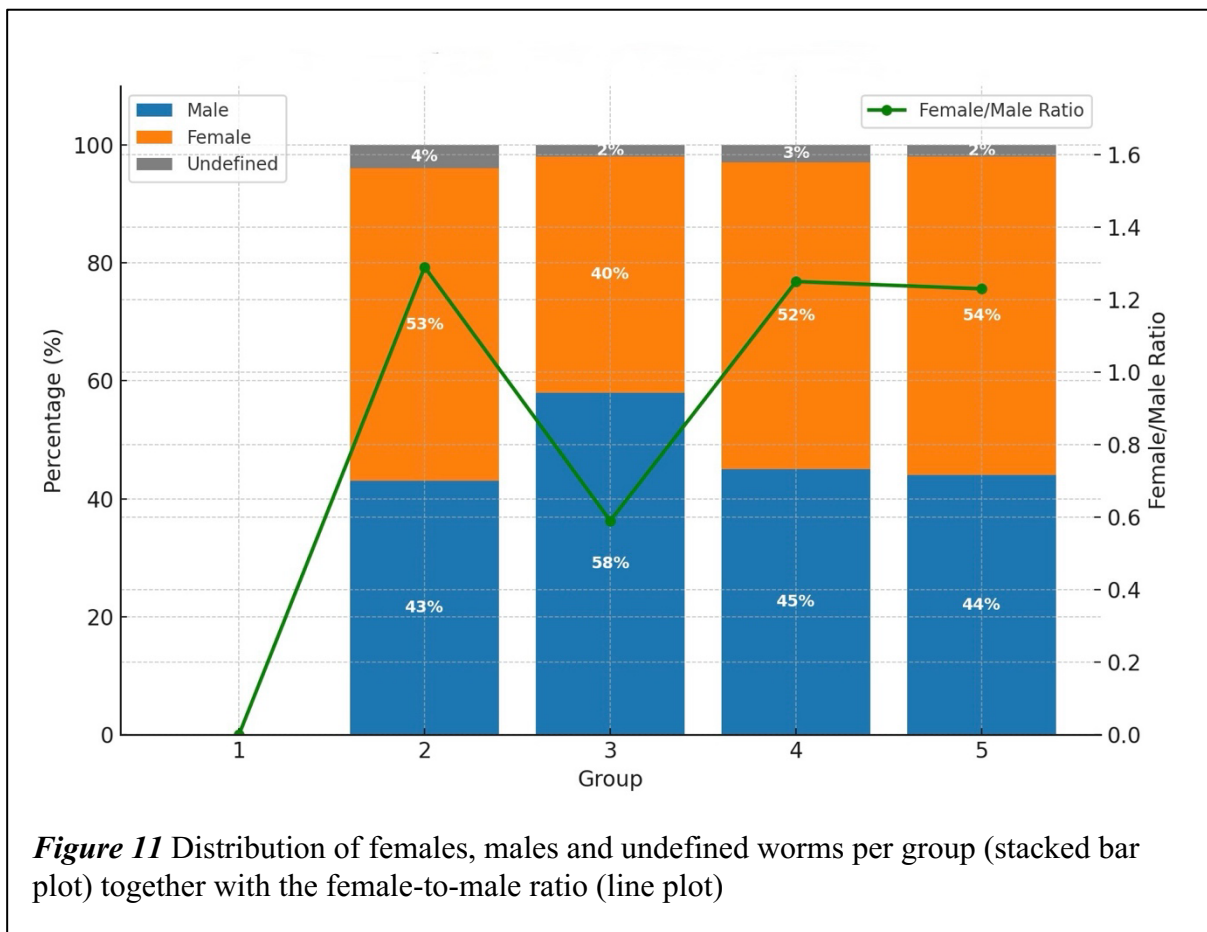
Table 3 Post-hoc-Sidak Tests between groups

Dependent Variable: Sidak (I) Group	Number of worms (J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower	95% Confidence Interval Upper
G2 No vaccination/ challenge	G3 Barbervax®/ challenge	1729.143*	376.564	0.001	649.90	2808.38
	G4 GErH11/ challenge	148.143	376.564	0.999	-931.10	1227.38
	G5 nGErH1/ challenge	-179.000	376.564	0.998	-1258.24	900.24
G3 Barbervax®/ challenge	G2 No vaccination/ challenge	-1729.143*	376.564	0.001	-2808.38	-649.90
	G4 GErH11/ challenge	-1581.000*	376.564	0.002	-2660.24	-501.76
	G5 nGErH1/ challenge	-1908.143*	376.564	0.000	-2987.38	-828.90
G4 GErH11/ challenge	G2 No vaccination/ challenge	-148.143	376.564	0.999	-1227.38	931.10
	G3 Barbervax®/ challenge	1581.000*	376.564	0.002	501.76	2660.24
	G5 nGErH1/ challenge	-327.143	376.564	0.950	-1406.38	752.10
G5 nGErH1/ challenge	G2 No vaccination/ challenge	179.000	376.564	0.998	-900.24	1258.24
	G3 Barbervax®/ challenge	1908.143*	376.564	0.000	828.90	2987.38
	G4 GErH11/ challenge	327.143	376.564	0.950	-752.10	1406.38

*. The mean difference is significant at the 0.05 level.

4.3. PROPORTION OF FEMALE AND MALE WORMS PER GROUP AS WELL AS FEMALE TO MALE RATIO PER GROUP

For each group, the proportions of female, male, and undefined worms were calculated, together with the total worm counts as well as the female to male ratio (**Fig.11**). Group 3 (Barbervax®) had the lowest proportion of female worms (40 %), with a female-to-male ratio of 0.59. In Group 2 (Positive), females represented 53 %, with a female-to-male ratio of 1.29. Groups 4 (GErH11) and 5 (nGErH11) showed higher proportions of female worms, with 52 % and 54 %, and female-to-male ratios of 1.25 and 1.23.



5. DISCUSSION

The observed variability in worm counts among the research groups reveals important differences in treatment outcomes. Group 2 (Positive) showed the widest range (628–3286), with a high median worm burden of 2587, indicating significant heterogeneity and susceptibility in the absence of intervention. These findings are consistent with prior findings demonstrating the high pathogenicity and reproductive potential of *H. contortus* under natural conditions (17).

In contrast, Group 3 (Barbervax[®]) showed a noticeably smaller range (107–1029) and the lowest median of all groups (353 worms), reflecting a consistent and effective reduction in worm burden. The significant reduction of 86.35 % in worm burden observed in Group 3 aligns with findings from the Moredun Research Institute, where vaccinated sheep exhibited an 86.93 % decrease in *H. contortus* worm burdens compared to unvaccinated controls. This underscores the efficacy of the Barbervax[®] vaccine in controlling *H. contortus* infections (21, 23, 24, 26).

Group 4 (GERH1) exhibited a broad range of worm burdens (1025–3332) with a median count of 1931 worms. This outcome indicates a partial protective effect, reflecting a 25.35 % reduction compared to the positive control. However, the substantial variability among individuals suggests inconsistent vaccine performance. Such partial protection aligns with earlier findings involving recombinant antigens. For example, González-Sánchez and coworkers (31) reported that immunization with recombinant rHc23 provided partial protection against *H. contortus* in lambs, though it was characterized by high individual variability and limited statistical significance. In this study, both glycoengineered and non-glycoengineered forms of the recombinant H11 antigen and GA1 were tested, aiming to assess whether post-translational modifications, particularly glycosylation, influence the antigen's immunogenicity and protective capacity. The H11 antigen, derived from the gut membrane of *H. contortus*, is classified as a hidden antigen (26, 27). In contrast, rHc23 is an somatic antigen, actively released by the parasite (6, 28).

These fundamental differences in antigen class, immune exposure, and functional roles limit the direct comparability of their protective efficacies (12).

Group 5 (nGErH11) displayed a narrower worm burden range (2025–3064), suggesting more consistent performance among individuals. However, the relatively high median burden (2232) and modest reduction of only 13.72 % compared to the positive control underscore its limited overall effectiveness. These findings align with previous research showing that recombinant versions of protective gut antigens, such as H11, often elicit weaker immune responses than their native counterparts (12, 29).

Despite its improved consistency, the efficacy remains a major challenge.

Comparing the outcomes of Group 4 (GErH11) and Group 5 (nGErH11) in reducing worm burden (25.35 % vs. 13.72 %) reveals a remarkable difference. Glycoengineering appears to positively influence the vaccine's ability to reduce worm burden.

These findings highlight that, while variability may suggest internal consistency, effectiveness is best evaluated through both dispersion and central tendency measures. Group 3 performed best on both metrics, while Groups 4 and 5 showed limited or inconsistent efficacy, supporting the idea that recombinant vaccines still require significant optimisation before they can rival existing control measures. ANOVA revealed statistically significant differences in worm burdens across groups ($p = 0.0001$), indicating that the treatments influenced infection levels. Post-hoc Sidak's tests showed no significant differences between Group 2 (positive control) and Group 4 (GErH11, $p = 0.99$) or Group 5 (nGErH11, $p = 0.998$), demonstrating that neither vaccine candidate significantly reduced worm burdens. However, both vaccinated groups exhibited modest numerical reductions in median worm burdens, suggesting a slight positive trend that may indicate partial biological efficacy.

In contrast, Group 3 (Barbervax[®]) exhibited significantly lower worm burdens compared to Group 4 (GErH11, $p = 0.002$) and Group 5 (nGErH11, $p = 0.0002$). These results demonstrate the efficacy of Barbervax[®] and underscore the limited impact of the experimental vaccine candidates. Similar patterns have been reported in trials with recombinant antigens, where promising *in vitro* immunogenicity did not always translate into field efficacy (13, 48). These findings emphasize the need for continued research into antigen discovery and optimization, as well as the development of improved delivery systems and adjuvant formulations.

Female *H. contortus* play a critical role in the pathogenicity of infection due to their high fecundity and blood consumption (32).

Therefore, shifts in female-to-male ratios offer an important metric for evaluating vaccine efficacy. In Group 2 (positive control), the ratio of 1.29 indicates a naturally female-biased population, consistent with high egg shedding and pathogenicity.

In Group 3 (Barbervax®), the ratio of 0.59 reflects a solid reduction in female prevalence, suggesting a robust effect on the most pathogenic subpopulation. A similar effect was found in the study of González-Sánchez et al. (6) using the recombinant rHc23 antigen vaccine candidate (somatic).

In Group 4 (G_{Er}H11), the ratio of 1.25 shows minimal reduction in female worms, demonstrating limited efficacy. Group 5 (nG_{Er}H11), the ratio of 1.23, closely resembling the positive control, further suggests insufficient vaccine-induced disruption of reproductive females. These findings underline the biological importance of targeting female worms in vaccine design.

Previous research has demonstrated that the protective efficacy of native gut antigens, such as H-gal-GP and H11, is partly due to their ability to impair the development and survival of female worms (21, 27). However, recombinant formulations of H11 have not yet matched this level of efficacy, possibly due to differences in glycosylation or epitope conformation (30).

The newly developed recombinant vaccine candidates against *H. contortus* represent a promising step toward more sustainable parasite control. To further enhance their effectiveness, future studies should explore the use of different adjuvant formulations and optimize antigen dosages per vaccine dose. Moreover, evaluating the vaccine in diverse sheep breeds and under field conditions including scenarios with natural co-infections involving other endoparasites will be critical for assessing its real-world efficacy and broader applicability.

6. CONCLUSION

This study aimed to evaluate the protective response of two novel recombinant vaccine candidates against *H. contortus* based on hidden H11 + GA1 antigens, more precisely, their impact on the abomasal worm burden in infected sheep. A closer inspection of the worm burden data of the clinical trial, range, median values, ANOVA results, and female-to-male ratio revealed clear differences in efficacy between the tested interventions. Two candidate vaccines were contrasted, one glyco-engineered (G_{Er}H11) and one non-glyco-engineered (nG_{Er}H11). Both were contrasted to a positive control group as well as to a commercially available vaccine (Barbervax[®]) based on native H11 + H-gal-GP antigens.

The Barbervax[®] group had a significant and statistically robust reduction in worm burden, with 86.36 % reduction over the positive control, consequently confirming the high efficacy and consistency of this commercial native antigen-based vaccine.

In contrast, no statistically significant decrease was achieved by either of the two recombinant vaccine candidates. The G_{Er}H11 vaccine provided a 25.36 % decrease, and the nG_{Er}H11 vaccine only provided a 13.72 % decrease compared to the positive control. Both groups had high variance for worm burdens and minimal impact on reducing the percentage of female worms, which are significant contributors to parasite transmission and pathogenicity.

These findings are contrary to the original hypothesis that the novel recombinant H11(hidden antigen) vaccine candidates would show significantly reduced *H. contortus* worm burden in sheep following the regimen of this trial. The findings point out some of the challenges of moving hidden recombinant antigen H11-constructs towards practical protective immunity, especially towards native gut antigen preparations. Further research is required to optimise antigen design, structural integrity, and formulation strategy to optimise immunogenicity and field efficacy.

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