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The effect of a novel vaccine candidate against *Haemonchus contortus* in sheep on the immunoglobulin G concentrations in the sheep sera

Diploma Thesis

submitted by

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Wien, den 29.09.2025

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Angaben zum Eigenteil:

1. Literaturrecherche
2. Praktische Tätigkeiten während der klinischen Versuchszeit im Stall: Klinische Untersuchung der Schafe, Probenentnahme: Blut, Speichel und Kot, Wiegen, Reinigung und Hygiene im Stall
3. Entnahme und Aufbereitung des Labmagens am Tag der Euthanasie
4. Zählung der Würmer und Bestimmen des Geschlechtes
5. Kot-Probenaufbereitung mittels Mini Flotac und Ei- Zählung im Parasitologischen Labor
6. Durchführung von ELISA im Labor
7. Daten Auswertung, Grafik Erstellung und Statistik

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1 Abstract:

Haemonchus contortus is an important pathogenic haematophagous nematode affecting small ruminants and South American camelids worldwide. A key challenge in treating *H. contortus* infections is the increasing resistance to antiparasitic drugs. This project aimed to develop a new glycoengineered recombinant anti-*H. contortus* vaccine. To assess its efficacy, a randomised, controlled vaccine trial was conducted. As part of this, immunoglobulin G (IgG) responses were analysed in blood samples from five trial groups, including positive and negative controls, the commercial Barbervax® vaccine (BVAX group) and two variations of the new vaccine candidate: the glycoengineered form (GEA group) and the non-glycoengineered form (NEA group). This diploma thesis specifically focused on the analysis of IgG responses within the framework of the trial. Blood samples from the jugular vein were taken at 24 events during the trial. To demonstrate the changes in the immunoglobulin G concentrations in the sheep sera, a *Haemonchus*-specific and a vaccine-specific ELISA was performed. The GEA and NEA groups showed a similar *Haemonchus*-specific IgG response to the Barbervax® vaccine. In the GEA group the antibody titer was not significantly higher than in the NEA group. However, the increase in IgG concentration after challenge was twice as high as that observed in the NEA group. Furthermore, egg shedding and worm burden were lower in the GEA group, which strongly indicates that glycoengineering improves the efficacy of the new vaccine candidate. Both the GEA and NEA groups appeared to be effective in inducing vaccine-specific IgG antibodies. Further studies are needed to optimise the glycoengineered vaccine against *H. contortus* by investigating the most effective recombinant antigen isoforms, vaccine doses and schedules of immunisation. A recombinant vaccine produced using glycoengineering in insect cells could reduce the reliance on anthelmintic drugs and thereby slow the progressive development of parasite resistance. Consequently, it may improve animal health and welfare and contribute to the development of vaccines against other nematodes.

2 Zusammenfassung:

Haemonchus contortus ist ein wichtiger pathogener, hämatophager Nematode, der weltweit kleine Wiederkäuer und Neuweltkamele infiziert. Eine zentrale Herausforderung bei der Behandlung von *H. contortus* Infektionen ist die zunehmende Resistenz gegen Anthelminthika. Daher ist die Entwicklung eines neuen Impfstoffes gegen *H. contortus* von großer Bedeutung. Ziel dieses Projekts war die Entwicklung eines neuen glykoengineerten rekombinanten Impfstoffs gegen *H. contortus*. Um dessen Wirksamkeit zu beurteilen, wurde eine randomisierte, kontrollierte Impfstoffstudie durchgeführt. Im Rahmen dieser Studie wurden die Immunglobulin-G-Reaktionen (IgG) in Blutproben von fünf Versuchsgruppen analysiert. Darunter positive und negative Kontrollen, der kommerzielle Impfstoff Barbervax® (BVAX-Gruppe) und zwei Varianten des neuen Impfstoffkandidaten: die glykoengineerte Form (GEA-Gruppe) und die nicht glykoengineerte Form (NEA-Gruppe). Diese Diplomarbeit konzentrierte sich speziell auf die Analyse der IgG-Reaktionen im Rahmen der Studie. Es wurde zu 24 Zeitpunkten Blutproben aus der Halsvene entnommen. Um die Veränderungen der Immunglobulin G Konzentrationen im Serum der Schafe nachzuweisen, wurden ein *Haemonchus*-spezifischer und ein Impfstoff-spezifischer ELISA durchgeführt. Die GEA und die NEA-Gruppe zeigten eine *Haemonchus*-spezifische IgG-Antwort, die mit der des Barbervax®-Impfstoffes vergleichbar war. In der GEA Gruppe war der Antikörpertiter nicht signifikant höher als in der NEA Gruppe, aber der Anstieg der IgG Konzentration war doppelt so hoch wie in der NEA Gruppe nach der Impfung. Darüber hinaus sind die Eiausscheidung und die Wurmbürden in der GEA Gruppe geringer, was stark darauf hindeutet, dass Glycoengineering die Wirksamkeit des neuen Impfstoffkandidaten verbessert. In beiden Gruppen, GEA und NEA, konnte eine individuelle Reaktion des Immunsystems durch die Bildung von impfstoffspezifischen IgG Antikörpern nach der Impfung nachgewiesen werden. Weitere Studien sind erforderlich, um den rekombinanten Impfstoff gegen *H. contortus* zu optimieren. Hierbei sollen die wirksamsten rekombinanten Antigen-Isoformen, die geeigneten Impfstoffdosen sowie die optimalen Impfzeitpunkte untersucht und angepasst werden. Ein rekombinanter Impfstoff, der mittels Glycoengineering in Insektenzellen hergestellt wird, hat das Potential, den Einsatz von Anthelminthika zu reduzieren, die zunehmende Resistenz zu verlangsamen und die Gesundheit sowie das Wohlergehen der Tiere zu verbessern.

3 Introduction:

Haemonchus contortus is one of the most important pathogenic nematodes affecting small ruminants and South American camelids worldwide (1, 2). It inhabits the abomasum, causing blood loss, poor body condition and in severe cases, death. Depending on the level of infection, blood loss can range from 0.2 to 0.6 litres per day (3, 4). Typical clinical signs of an infection with *H. contortus* include weight loss, decreased milk production, weakness, anaemia and intermandibular and cervical oedema due to hypoproteinaemia, a so called 'bottle jaw' (1).

The life cycle of *H. contortus* has two main parts, a free-living stage and a parasitic stage. The eggs develop in the environment in two steps from L1 into L2 and then to the infective L3, within seven days, depending on environmental conditions. During grazing at the pasture, small ruminants and South American camelids become infected by the parasite. The larvae penetrate the mucosa of the abomasum and develop into the L4 stage. At this stage, hypobiosis may occur depending on the seasonal changes, changes in temperature, humidity, host hormones and the host immune status (5–9). This stage can last for several months, serving as a reservoir and causing further contamination of new pastures. Especially in spring when the weather gets warm and wet after lambing season, the larvae leave the mucosa and mature into an adult worm that copulates. This leads to an increase in egg shedding and new infections in young sheep, which allows the cycle to begin again (10, 11). The main pathomechanism of *H. contortus* occurs as a result of mucosal lesions and haematophagy caused by larvae and adult worms. This can severely weaken the body and makes it more susceptible to secondary infection by other pathogens.

The characteristic appearance of *H. contortus*, with the intestine and uterus of the female worm intertwined, is why it is also known as the 'barber-pole' worm. This intertwined structure is an easy macroscopic property to identify female worms. In comparison, male worms are smaller and possess a bursa with two lateral lobes on their posterior end. A single female worm can lay approximately 5,000 eggs per day, depending on the micro-environment of the abomasum (12, 13). The complete life cycle, from ingestion of larvae to the excretion of eggs in faeces and development into L3 larvae takes three to five weeks depending on the environmental condition. The prepatent period varies between 15 and 21 days (11).

An infection with *H. contortus* is often suspected based on typical clinical signs such as anaemia, reduced general behaviour, decreased food intake and weight loss. Alternatively, it can be assessed using scoring systems such as FAMACHA® or BCS Score (1, 14, 15).

However, clinical parameters showed only weak correlations with faecal egg counts, and it was confirmed that a minority of the flock is responsible for the majority of pasture contamination with trichostrongyloid eggs (16). Therefore, effective control and diagnostic strategies are essential to identify animals that would benefit the most from treatment and to manage parasite infections sustainably.

One approach involves collecting faeces and performing the Mini-FLOTAC technique to count the eggs under a light microscope, thereby determining the parasitic load. However, faecal egg counts do not correlate well with worm burden. But identifying high egg shedders makes it possible to treat them selectively. This reduces pasture contamination and minimises infection pressure for susceptible individuals. In order to prevent clinically ill sheep and slow the development of anthelmintic resistance, a target-selective deworming and a good farm management are recommended. This includes grouping sheep by age, strip grazing, additional hay feeding and long pasture breaks (17). Anthelmintic drugs currently used for sheep include benzimidazoles (e.g., albendazole, fenbendazole), macrocyclic lactones (e.g., ivermectin, moxidectin), salicylanilids (e.g. closantel) and amino-acetonitrile derivatives (e.g. monepantel) (18). For target-selective treatment the general behaviour and body condition score can be used as markers to determine the timing of treatment (19). Also the colour of the conjunctival mucosa can be assessed using the FAMACHA® scoring system (20). If the score is three, deworming should be considered. A score of four or five indicates that the sheep is suffering from anaemia due to blood sucking parasites such as *H. contortus*. In this case the sheep should be treated accordingly. During the grazing season the sheep should be rechecked every two weeks (21). A tool to detect secondary infections is the so called 'Dag score', which evaluates the quantity and distribution of faecal matter in the perianal area (19).

The major advantages of implementing farm-specific monitoring and target-selective treatment strategies include reduced egg shedding, minimised infective pressure on the pasture and reduced treatment costs. It also enable to identify sheep that can cope with infection and thereby progressing their resilience against *H. contortus*. This concept therefore lowers the selection pressure for anthelmintic-resistant of the parasite and helps to slow the development of anthelmintic resistance (20). Many farmers already implement several of these management strategies in order to prevent severe infections in their livestock. However, these measures alone are insufficient to fully protect animal health and prevent economic losses. With the increasing prevalence of anthelmintic resistance, the development of a vaccine against *H. contortus* would represent an effective tool to complement and improve prevention strategies.

The infection with *H. contortus* in sheep triggers cellular, humoral and inflammatory mechanisms of the immune system. The innate immune system is activated after the first infection, involving macrophages, dendritic cells, and natural killer cells. Cytokines, particularly IL-4, are released and activate B- and T-cells of the acquired immune system, which plays a major role in establishing a specific immune response. The recognised antigens stimulate T-helper cells to secrete interleukins that, in turn, stimulate B-cells to produce and secrete antibodies (IgE, IgG, IgA) (22–24). These antibodies bind to parasitic antigens, forming immune complexes that are recognised by mast cells, eosinophils, and neutrophils, which then release the contents of their granules, including histamine, prostaglandins and leukotrienes. The release of these mediators causes inflammation, increases mucus production, stimulates contraction of smooth musculature, and thereby contributes to parasite elimination or death (25–28).

Immunoglobulin G is one of the main systemic antibodies involved in helminth infections (29). Only a few studies have been investigated IgG levels in the sera of sheep infected with *H. contortus*. It has been shown that the immune response is age-dependent. In previously infected Manchego lambs aged weeks, no increase in *Haemonchus*-specific serum antibodies was observed, whereas a weak increase was detected in challenged 6.5-month-old lambs. Furthermore, the IgG response against larval antigens was found to be higher than that against soluble proteins of adult worms (30). Overall, high IgG titers often correlate with improved protection of sheep against *H. contortus* (31). A key effector in anti-helminth immunity is immunoglobulin E (IgE), which triggers mast cells and activates eosinophils responsible for parasite expulsion (32). IgE levels have been shown to correlate with resistance in naturally immune sheep (33, 34).

A key challenge in preventing and treating *H. contortus* infections is the growing resistance to antiparasitic drugs (33–37). Currently, the commercial vaccine Barbervax® is available in Australia. This vaccine is not accessible in many countries. Thus, it is urgently needed to develop a safe, well-defined, recombinant vaccine which can be produced cost-effectively under controlled conditions containing defined antigens.

To meet this need many different potential vaccine targets were identified and evaluated in in-vivo trials (35). There were a few approaches to develop an alternative vaccine against *H. contortus*. In the past many different vaccine candidates were tested by using various vaccination strategies including recombinant subunit vaccines, DNA vaccines, and protein

vaccines (36–39). However, only the combinations of gut-derived protein-enriched H-gal-GP protease and H11 fractions in the native, folded and glycosylated form of *H. contortus* showed significant reductions in worm burden, faecal egg count and IgG levels. This indicates that vaccine efficacy is associated with *H. contortus*-specific serum IgG levels, triggered by the native form of the antigens. (40, 41).

The Barbervax® vaccine is produced directly from adult worms harvested from the stomach of slaughtered sheep. It contains of protein cleaving enzymes H11 and H-gal-GP which are located on the surface of the intestine of the adult worm (42). Therefore, both antigens are not accessible to the host's immune system during an infection. Trials using Barbervax® revealed that anti-H11 IgG damages the gut of *H. contortus*. Consequently, it reduces the blood-feeding activity of the adult worm (31, 41). Cause of this “hidden” antigens, naturally infected sheep does not boost the immune system which leads to the assumption that repeated vaccination is needed to keep the antibody titers high (43).

To produce recombinant H11 antigens, conventional expression platforms or the nematode *Caenorhabditis elegans* have previously been used (44–47). However, these antigens were not sufficiently effective in reducing the worm burden or faecal egg counts in sheep (35). Naturally, H11 is glycosylated, which is considered crucial for maintaining its antigenicity (46, 48). Building on this knowledge, a novel recombinant vaccine against *H. contortus* has been developed using five soluble recombinant proteins (H11, H11-1, H11-2, H11-4 and GA1) containing nematode-type glycan modifications produced in High Five insect cells (49). To evaluate whether this novel vaccine candidate is safe, immunogenic, and protective, a randomised controlled vaccine trial was carried out (50).

The present study aims to detect and compare immunoglobulin G responses in blood samples from five trial groups. The positive control group was unvaccinated, but challenged with *H. contortus* L3 while the negative control group was unvaccinated and uninfected. The three vaccines tested in this study included the commercial Barbervax® vaccine (BVAX group), and two variations of the new vaccine candidates: the glycoengineered form (GEA group) and the non-glycoengineered form (NEA group).

It is hypothesised that the novel vaccine candidates, followed by challenge with *H. contortus*, will increase the immunoglobulin G concentrations compared to control groups. It is also hypothesised that the glycoengineered form will trigger a stronger parasite-specific immune response and increase of immunoglobulin G concentrations in the sheep sera.

4 Results and Discussion:

4.1 *Haemonchus*-specific immunoglobulin G response:

The *Haemonchus*-specific ELISA results of NEA, GEA and BVAX group for immunoglobulin G shows higher OD-values, than in the positive and negative control groups. The difference was statistically significant with a p-value of 0.01 to 0.001 (see Figure 1 and Table 1). The GEA and NEA groups elicited a *Haemonchus*-specific IgG response comparable to that induced by the Barbervax® vaccine. It can be assumed that the OD-values represent the immunoglobulin G titers in the sera. Therefore, all three vaccines induce parasite-specific antibodies after vaccination and challenge. In comparison with the GEA and NEA groups, the BVAX group showed nearly a two-fold increase in IgG antibody concentration after the third vaccination, rising from an OD of 0,6188 to OD 1,1003. The immunoglobulin G concentrations rapidly increased in all groups one week after the challenge with 5,000 *H. contortus* L3 larvae (Figure 1). An increase in IgG titers indicates a strong parasite-specific immune response and is often associated with improved protection (31). However, it is assumed that some damaged worms were introduced during the challenge, leading to the presentation of parasite antigens also in the unvaccinated group.

The delayed onset of the antibody response appears to be related to the prepatent period of the nematode (51). Two weeks after the challenge, the IgG concentration peaked. The relative increase in antibodies levels compared with the concentrations on the day of challenge was 74% in the BVAX group, 66% in the GEA group and 32% in the NEA group, and 35% in the positive control group. Thus, the NEA group exhibited the lowest response, even below that of the positive control, indicating markedly reduced immunogenicity. At this time point, the immunological memory generated by vaccination reached peak antibody production. The similar pattern of IgG concentration in the GEA and BVAX groups during the trial, characterised by a sharp increase after challenge, may be associated with lower egg shedding and worm burden, and thus with the high efficacy shown in these two groups (16).

Despite that there was no statistically significant difference in the IgG concentration among the NEA, GEA and BVAX groups ($p > 0.05$), overall OD values were higher in the BVAX group. However, the BVAX group showed lower egg shedding and worm burden than both the GEA and NEA groups. The egg shedding and worm burden were also lower in the GEA group compared to NEA, which strongly indicates that glycoengineering improves the efficacy of the new vaccine candidate (50, 35).

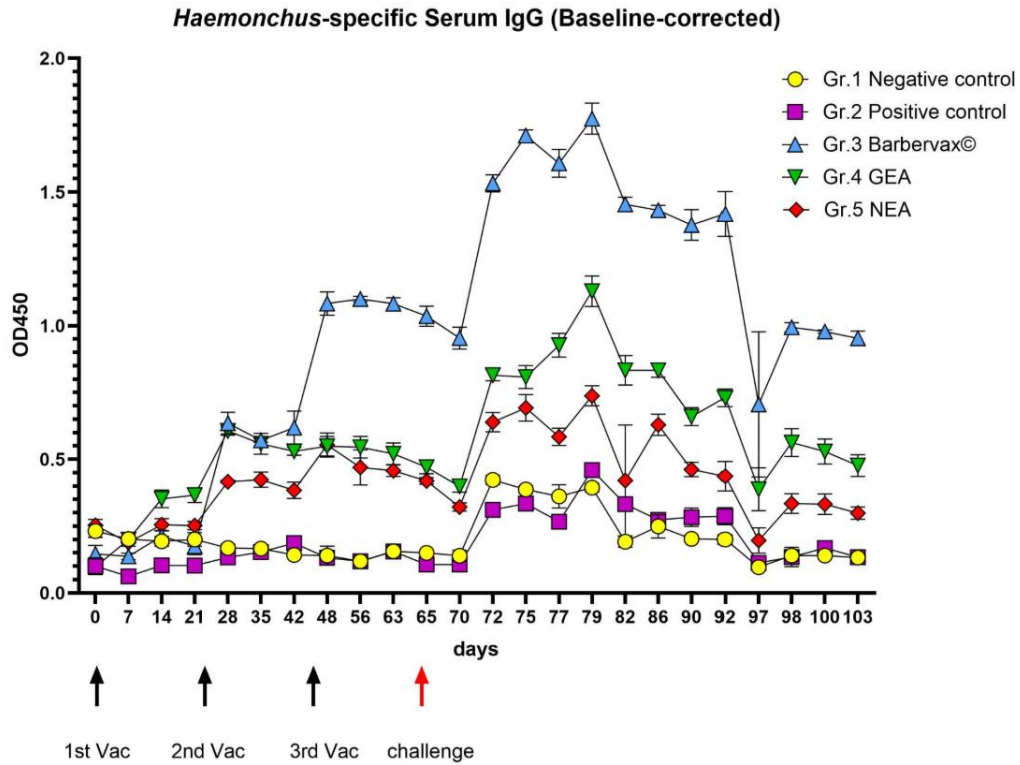


Figure 1: Baseline-corrected *Haemonchus*-specific serum immunoglobulin G ELISA results. The figure shows that all three vaccine groups: BVAX, GEA and NEA respond to the *Haemonchus*-specific coating in the ELISA after vaccination and challenge. The antibody levels are significantly higher than in the positive and negative control groups (p-value: 0.01–0.001). There are no statistically significant differences between the BVAX, GEA and the NEA groups (p-value: >0.05) shown in table 1.

Table 1: Statistical analysis of the *Haemonchus*-specific immunoglobulin G ELISA data of all 24 time points. A Shapiro-Wilk test was performed to check for normal distribution. A Kruskal-Wallis test was then performed to determine significance. The table shows the results of the Dunn's multiple comparison test and the corresponding p-value interpretation.

Group comparison

1 vs. 2	ns					p-value:	
1 vs. 3	***	2 vs. 3	***	3 vs. 4	ns	**	≤0.01
1 vs. 4	***	2 vs. 4	***	3 vs. 5	ns	***	≤0.001
1 vs. 5	**	2 vs. 5	***	4 vs. 5	ns	ns	>0.05

4.2 Vaccine-specific immunoglobulin G response

After performing the vaccine-specific immunoglobulin G ELISA, the results show high individual variation in the immune response to the new vaccination candidates, as it is shown for GEA in Figure 2. This variation in immune responses to the vaccine can be attributed to intrinsic host parameters such as age, sex and genetics, as well as extrinsic environmental, behavioural, and nutritional factors (52, 28, 53). All the sheep used in the trial came from the same farmer, were of a similar age, male and were fed the same hay. However, these individual differences may have contributed to the large error bars observed in Figure 3, where the mean results are shown. For the ELISA assays, plates were coated with either GEA or NEA antigens to assess vaccine-specific responses. In contrast, plates for the BVAX, positive and negative control groups were coated with soluble BVAX antigens. Due to these differences in plate coating, OD values from the BVAX coated plates cannot be directly compared to the GEA and NEA group. It can be assumed that ELISA OD values correlates with antibody titres in the sera.

As shown in Figure 3A and 3B, antibody concentrations in the sera of sheep from the GEA and NEA groups increased rapidly, indicating that both new vaccine candidates efficiently induced antigen-specific antibody responses. The GEA group exhibited higher OD values than the NEA group after the second and third vaccinations, as well as after challenge, suggesting that the glycoengineered form elicited a more robust IgG response.

The comparatively low titers of vaccine-specific antibodies observed in the BVAX group may be explained by reduced antigen-coating efficiency and potential cross-reactivity. Furthermore, the Barbervax® vaccine is composed of several different antigens presented at varying concentrations, which cannot be standardised during ELISA coating (Figure 3A).

ELISA Results: Vaccine-specific IgG

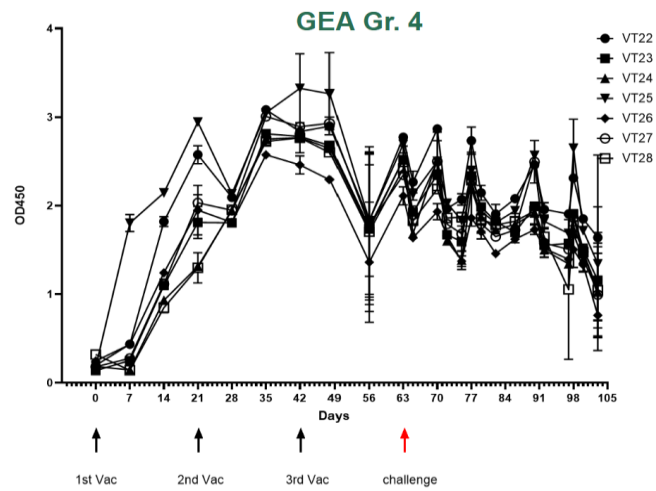


Figure 2: Vaccine-specific immunoglobulin G response of all individuals in the GEA group (VT22-VT28). The results demonstrate large differences in the immune response of each individual at each time point. (Graph: Isabella Adduci)

ELISA Results: Vaccine-specific IgG

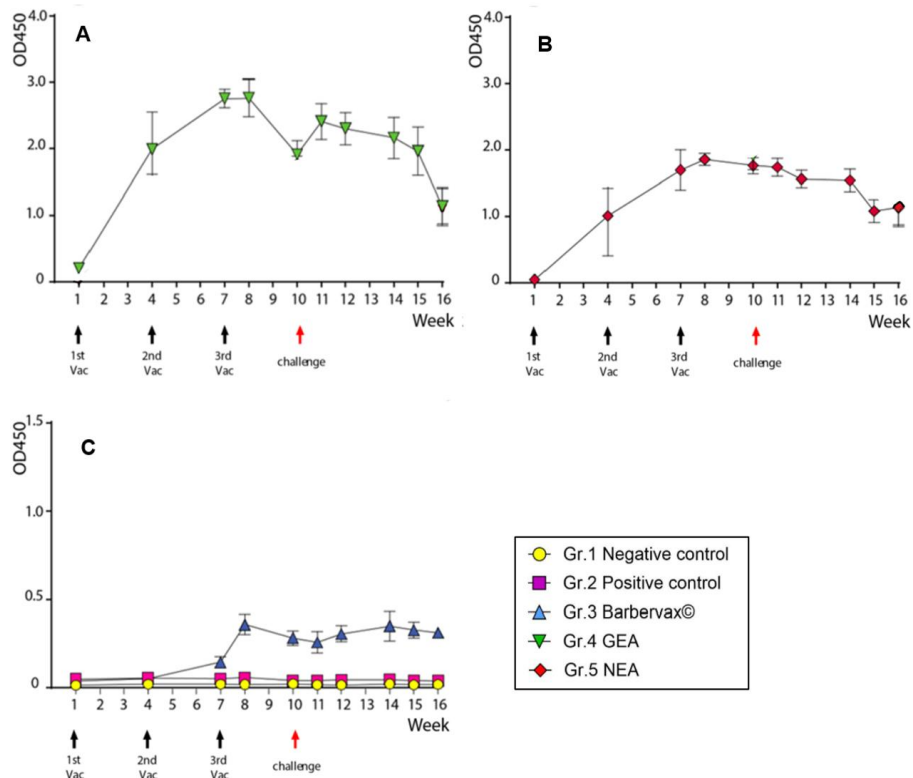


Figure 3: Vaccine-specific immunoglobulin G response. (A) GEA-coated plates with the GEA group; (B) NEA-coated plate with the NEA group; (C) Barbervax®-coated plates with the BVAX, positive and negative control groups. (Graph: Isabella Adduci)

This vaccine trial provided valuable efficacy data and clearly demonstrated the importance of glycoengineering in enhancing the immune response, increasing the IgG concentration and therefore improved protection against *H. contortus*.

Despite these promising results, the trial has several limitations. The 3R principle was used for the calculation of the sample size, since a larger number of sheep would not be beneficial at this stage of vaccine development, only seven individuals in each group were included in the trial (54). This is a small sample size to show every possible individual immune response. Therefore the data are more indicative of trends. Furthermore, all sheep remained clinically healthy throughout the trial, suggesting that the L3 challenge dose may have been too low to induce a full infection. The ELISA was performed by coating 96-well plates with either BVAX or soluble proteins derived from adult *H. contortus*. Different antigen concentrations were required to minimise cross-reactivity, and for BVAX in particular it remains unclear how the antigens bind to the plate or in what quantities, meaning deviations in OD values are possible. Although all laboratory personnel were well trained and strictly adhered to the protocol, some degree of bias cannot be excluded.

In addition to the vaccine, the used adjuvant plays a critical role in inducing and preparing the immune response for potential infection (55). A recent study evaluated a vaccine platform for extended antigen release (VPEAR) which is designed to initiate and maintain long-term immunity in sheep following a single immunisation (56).

Further studies are required to optimise the glycoengineered vaccine, including investigation of the most effective recombinant antigen isoforms, vaccine doses, and immunisation schedules. A recombinant vaccine produced in insect cells using glycoengineering has the potential to reduce the use of anthelmintic drugs, slow the development of resistance, and improve animal health and welfare and help to develop vaccines against other nematodes.

5 Publication:

<https://doi.org/10.1038/s41541-025-01249-z>

Safety and efficacy of a novel glycoengineered recombinant vaccine candidate against *Haemonchus contortus* in sheep



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Haemonchus contortus is a highly pathogenic nematode in small ruminants, causing major production losses and animal welfare concerns. With increasing anthelmintic resistance, vaccination offers a more sustainable control strategy. This study evaluated a novel glycoengineered vaccine produced in Hi5 insect cells in a randomized, controlled trial including Jura × Lacaune sheep. Animals received either Barbervax® (BVAX), the glycoengineered vaccine (GEA) composed of five antigens (H11, H11-1, H11-2, H11-4 and GA1), its non-glycoengineered counterpart (NEA), or served as controls. Over 16 weeks, clinical, immunological, and parasitological data were collected. GEA reduced fecal egg counts by 81.09% and worm burden by 25.36%, showing a lower degree of anemia compared to NEA and control. All vaccinated groups exhibited elevated IgG/IgE responses. The results underscore the importance of glycoengineering in achieving protective immunity against *H. contortus*, supporting the development of highly effective recombinant vaccines against this and other parasitic worms in the future.

Haemonchus contortus is the most pathogenic gastrointestinal nematode in small ruminants and South American camelids. Infection cause significant production losses (decreased weight gain and fertility as well as loss of young stock) and threatens animal welfare worldwide, especially in tropical and subtropical areas^{1–6}. As a single worm ingests up to 50 µl of blood per day, even moderate infections can cause severe blood loss, resulting in anemia and hypoproteinemia. In severe cases, even death may occur^{5,7}. Currently, parasite control relies primarily on the administration of anthelmintic drugs, which have several disadvantages. For instance, increasing prevalence of anthelmintic-resistant worms, long withdrawal periods for meat and milk and unwanted ecotoxicological effects^{8–10}. Due to a lack of treatment response of resistant *H. contortus*, clinical disease will progress in affected hosts^{10–13}.

In contrast, vaccination is a promising strategy to control the parasite in susceptible domestic animal species. In the past, a repertoire of potential vaccine targets, including somatic and secreted antigens, was identified and

evaluated in in vivo trials¹⁴. Among the evaluated protein-based vaccine candidates, the H11 aminopeptidases and the H-gal-GP protease complex showed the highest efficacy^{15,16}. Sheep vaccinated with either antigens showed significant reductions in fecal egg shedding of 72%–93% and of 68%–69% in worm burden^{17,18}. Both H11 and H-gal-GP are “hidden” proteins, located in the intestine of adult worms, and are not naturally exposed to the host’s immune system during worm infection. Thus, a vaccine response to these antigens is not boosted by infection. Therefore, a repeated immunization is needed to maintain a protective effect during the grazing season¹⁹. H11 and H-gal-GP are major components of Barbervax®, which was licensed in Australia in 2014, but is available only in a few countries.

To meet local biosecurity regulations, Barbervax® is manufactured at the Albany Laboratory of the Department of Agriculture and Food in Western Australia from adult *Haemonchus* worms harvested from the stomach of donor sheep after slaughter on an industrial scale. One production step of

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Barbervax® involves mechanical disruption of the worms, by which 480 mg of antigen can be obtained from 90 g of worms^{20,21}. For commercial-scale production, machines were implemented to open the stomachs and harvest the worms. The antigens are then isolated and formulated as a vaccine²⁰. This approach is currently the only way to obtain adult worms for antigen preparation, as in vitro parasite culture systems for the propagation and multiplication of *H. contortus* are not yet been established²². Attempts to produce recombinant H11 vaccine antigens, using conventional expression platforms or the free-living nematode *Caenorhabditis elegans*, have been reported previously^{23–26}. The resulting products were unable to reach a satisfactory efficacy regarding the reduction of worm burden and/or fecal egg count (FEC)¹⁴. H11 proteins are naturally glycosylated, carrying very distinct N-glycan structures compared to those found in mammals. Such glycan modifications are often considered crucial for maintaining the antigenicity^{25,27}. To address this, a novel glycoengineered recombinant vaccine was designed that incorporates nematode-type glycan modifications and enhances solubility²⁸. This novel recombinant vaccine contains five soluble proteins (H11, H11-1, H11-2, H11-4 and GA1) produced using Hi5 insect cells.

The objectives of the present study were to compare hematological and parasitological parameters between sheep vaccinated with the novel recombinant vaccines (GEA/NEA) or Barbervax® (BVAX), an unvaccinated/challenged positive (POS), and an uninfected, unvaccinated negative (NEG) control group. It was hypothesized that vaccination of sheep with the novel vaccine would result in reduced anemia, a significant decrease in fecal egg shedding and abomasal worm burden and an elevated concentration of specific immunoglobulins in comparison to the POS group. Furthermore, we expected results to be comparable or better than those seen in sheep of the BVAX group and that the level of protection was higher in sheep vaccinated with the glycoengineered form (GEA group) of the vaccine compared to the non-glycoengineered form (NEA group).

Results

Clinical assessment

Following the challenge ($\geq$ week 10), the clinical anemia scores (FAMACHA®) of the BVAX, GEA, NEA and NEG groups were lower than

those of the POS group ($p < 0.01$). Mixed-model analysis revealed a mean FAMACHA® score of 2.42 for NEG (95%CI = 2.26; 2.59), 3.46 for POS (95%CI = 3.29; 3.63), 2.27 for BVAX (95%CI = 2.10; 2.44), 2.31 for GEA (95%CI = 2.14; 2.48) and 2.17 for NEA group (95%CI = 2.00; 2.33) (Fig. 1).

In addition to the FAMACHA® score, changes in daily body weight gain (DBWG) were monitored. Prior to L3 challenge (week 1 to 9; 63 days in total) no differences in DBWG between the groups were observed among the groups, with average gains ranging from 95 g to 222 g (Fig. 2; $p = 0.87$). After challenge ($\geq$ week 10; 40 days in total), a significant difference in DBWG was detected between the BVAX and NEG groups, with BVAX sheep gaining less weight (Fig. 2; $p = 0.02$). All other groups showed a numerical but no statistical significant increase in DBWG. The NEG group gained a median of 213 g/d (min of 138 g, max of 238 g), the POS 150 g/d (min of 125 g, max of 188 g), the BVAX 138 g/d (min of 88 g, max of 138 g), the GEA 150 g/d (min of 113 g, max of 225 g) and the NEA 175 g/d (min of 100 g, max of 225 g).

Both GEA and Barbervax® improve the values of hematological parameters

Packed cell volume (PCV), erythrocytes per milliliter and hemoglobin concentrations were measured as indicators of potential blood loss and anemia. All three parameters were lowest in the POS group and highest in the NEG group after challenge (Fig. 3 and Table 1).

Globulin and total protein concentrations were higher in the GEA group in comparison to the POS group depending on the time point after challenge (Fig. 4). The BVAX and GEA groups showed higher globulin concentrations (calculated as total protein minus albumin) than the NEA group throughout the entire period. Total protein levels did not differ between groups at weeks 8 ($p = 0.08$), week 9 ($p = 0.06$) and week 10 ($p = 0.06$). One week post challenge (week 11), the POS group showed lower total protein levels than the BVAX ($p < 0.01$), GEA ($p < 0.01$) and NEA ($p = 0.02$) groups. In week 12, there were no differences between the groups. In week 13, the POS group showed different total protein concentrations compared to the NEG ($p < 0.01$) and BVAX ($p < 0.01$) groups. The NEA group showed significantly lower total protein than the NEG group. In week

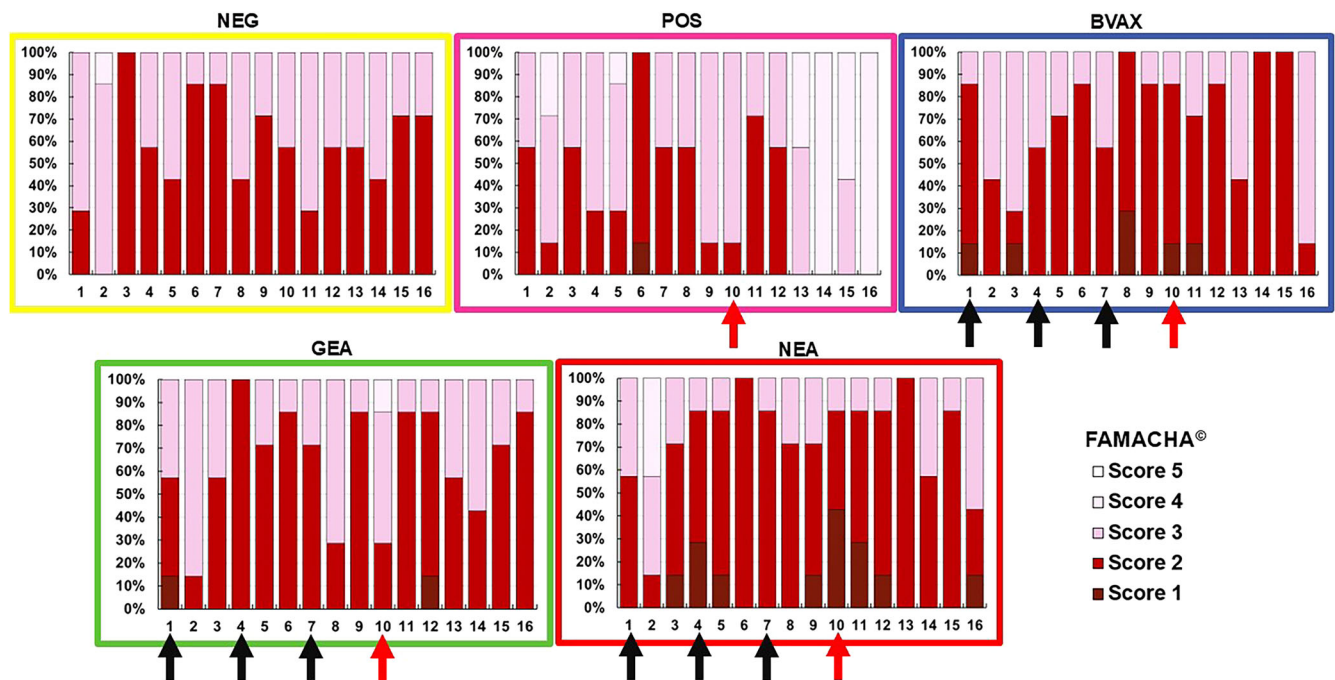


Fig. 1 | FAMACHA® scores in the five study groups (n = 7 sheep/group) during the 16-week study period (x-axis). The five graphs show the FAMACHA® results for the NEG, the POS, the BVAX, GEA and NEA groups. Each column shows the relative percentage of the FAMACHA® score of the seven sheep within each group

per study week. Black arrows highlight the 1st (week 1), 2nd (week 4) and 3rd (week 7) vaccination with either vaccine. The red arrow indicates the timepoint of challenge with 5000 L3 *H. contortus* (week 10).

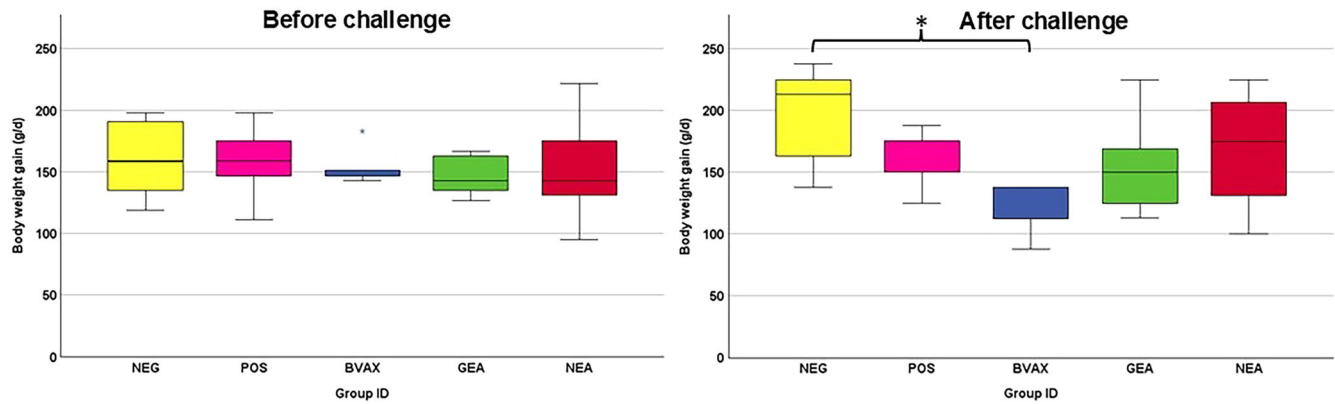


Fig. 2 | Overview on the daily body weight gain (DBWG; g/d) before and after challenge. There was no difference between the DBWG before challenge but after challenge the BVAX group showed a significantly lower DBWG.

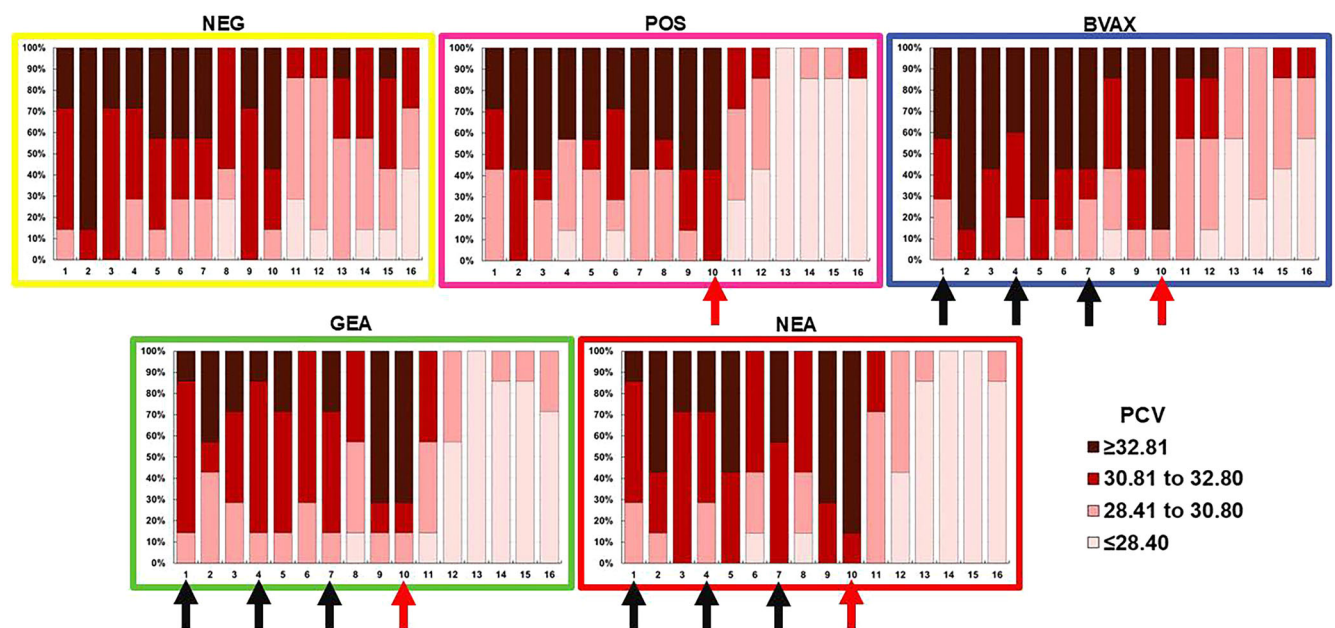


Fig. 3 | Packed cell volume (PCV [%]) in the five study groups over the study period of 16 weeks (x-axis). Each column shows the relative percentage of the PCV of the seven sheep within each group per study week. Black arrows highlight the 1st (week 1), 2nd (week 4) and 3rd (week 7) vaccination with either vaccine. The red arrow indicates the timepoint of challenge with 5000 L3 *H. contortus* (week 10).

Each column shows the relative percentage of the results of the PCV of the seven sheep within the group in study week 1 to study week 16. The black arrows highlight the 1st (week 1), 2nd (week 4) and 3rd (week 7) vaccination with either BVAX, GEA or NEA. The red arrows highlight week 10, when the sheep were challenged with 5000 L3 *H. contortus*.

14, both BVAX and GEA groups had higher total protein concentrations than POS ($p = 0.01$). In week 15, BVAX, GEA and NEG groups maintained higher total protein concentrations than the POS ($p = 0.01$). Additionally, week 15 showed differences between POS and NEG ($p = 0.04$) and between BVAX and POS ($p = 0.05$).

The results showed statistically significant differences in total protein from week 10 onward (after challenge) between the GEA group (mean of 5.95 g/dl) and the POS group (mean of 5.58 g/dl), as well as between GEA and NEA (mean of 5.58 g/dl) (Table 2). Albumin concentrations did not differ between the BVAX and GEA groups. However, albumin concentration was higher in the BVAX group (mean of 3.76 g/dl) compared to the NEA group (mean of 3.52 g/dl) ($p \leq 0.05$) (Table 2). No differences in albumin concentrations occurred at weeks 10 ($p = 0.48$) and 11 ($p = 0.79$). In week 12, the NEA group differed significantly from the NEG group ($p = 0.02$). In week 13, there was a difference between the NEA ($p < 0.01$) and GEA ($p = 0.05$) and the NEG, the POS and the NEG

group ($p = 0.01$). There were no differences in week 14. In weeks 15 and 16, significant differences appeared between the POS and the NEG ($p = 0.01$ and $p = 0.05$, respectively), and between NEA and NEG group ($p = 0.02$ for both weeks).

The groups differed in the number of segmented neutrophils, eosinophils, basophils, and lymphocytes. Statistically significant differences appeared in the number of eosinophils, with the BVAX group showing the highest numbers throughout the trial. The relative distribution of the white blood cells per group over the period of 16 weeks is shown in Fig. 5. In week 1 ($p = 0.37$), week 2 ($p = 0.07$), week 3 ($p = 0.24$) and week 4 ($p = 0.54$), eosinophil percentage did not differ significantly between the five groups. Notably, all vaccinated groups exhibited a marked increase in eosinophils after the second vaccination (week 5 ($p = 0.00$), week 6 ($p = 0.01$), week 7 ($p = 0.04$), week 8 ($p = 0.01$). In week 9 ($p = 0.08$) and 10 ($p = 0.43$), eosinophil percentages again showed no differences. Two weeks after the challenge infection (week 12), eosinophil counts increased in the POS,

BVAX and GEA groups but not in the NEA group. Statistical analysis revealed that the GEA group had the highest lymphocyte count (4679.40/ μ l), while the BVAX group had the highest eosinophil (507.17/ μ l) and basophil (52.75/ μ l) counts (Table 3).

All vaccines show similar impact on clinical parameters after the 1st, 2nd, and 3rd vaccination

Body temperature was measured before vaccination, and with a mean of $39.37^{\circ}\text{C} \pm 0.23^{\circ}\text{C}$, there was no difference between the groups ($p = 0.24$). All sheep showed a bright and alert appearance and good appetite after vaccinations. After the second vaccination differences were seen on the day

Table 1 | Differences between the five groups (indicated by superscript letters) in hematological parameters after challenge ($\geq$ week 10)

Group ID	Mean [95%CI]	Overall p value
Erythrocytes (*10 ⁶ /μl)		
NEG ^{a**} ,b ^{**} ,c ^{**}	9.95 [9.62; 10.28]	< 0.01
POS ^{a**} ,d ^{**} ,e ^{**} ,f [*]	7.38 [7.05; 7.70]	
BVAX ^{d**} ,g [*] ,h ^{**}	9.38 [9.05; 9.71]	
GEA ^{b**} ,e ^{**} ,g [*]	8.68 [8.35; 9.01]	
NEA ^{c**} ,f [*] ,h ^{**}	8.29 [7.96; 8.62]	
Packed cell volume (%)		
NEG ^{a**} ,b ^{**} ,c ^{**}	30.22 [29.36; 31.07]	< 0.01
POS ^{a**} ,d ^{**} ,e ^{**} ,f [*]	24.34 [23.48; 25.19]	
BVAX ^{d**} ,g [*] ,h ^{**}	29.10 [28.24; 29.96]	
GEA ^{b**} ,e ^{**} ,g [*]	26.77 [25.92; 27.63]	
NEA ^{c**} ,f [*] ,h ^{**}	26.11 [25.25; 26.96]	
Hemoglobin (g/dl)		
NEG ^{a**} ,b ^{**} ,c ^{**}	10.43 [10.12; 10.73]	< 0.01
POS ^{a**} ,d ^{**} ,e ^{**} ,f [*]	7.84 [7.54; 8.15]	
BVAX ^{d**} ,g [*] ,h ^{**}	9.84 [9.53; 10.15]	
GEA ^{b**} ,e ^{**} ,g [*]	9.03 [8.72; 9.34]	
NEA ^{c**} ,f [*] ,h ^{**}	8.53 [8.22; 8.83]	

Superscript letters highlighted with * indicate p values ≤ 0.05 , those with ** indicate p values < 0.01 for differences between groups with the same letter. The p values in the right column indicate differences between the groups overall.

of vaccination (Table 4). At the day of the second vaccination one sheep vaccinated with GEA showed a body temperature of 39.6°C 6 h after vaccination and 41.5°C 8.5 h post vaccination and a slightly reduced demeanor.

Induration, lymph node enlargement, swelling and pain was recorded in all experimental groups with differing degrees after each vaccination (Supplementary Tables 1–4).

The glycoengineered vaccine reduces egg shedding and eggs per female worm

To evaluate vaccine efficacy against infections with *H. contortus* fecal egg counts (displayed as eggs per gram of feces = EpG) and worm burden were assessed. The reduction of egg shedding was 98.32% for BVAX, 81.09% for GEA and 32.72% for NEA in comparison to the POS group (Fig. 6). The BVAX group showed lower egg counts than the POS ($p < 0.01$) and the NEA groups ($p = 0.02$). Egg counts did not differ between the BVAX and GEA ($p = 0.33$). Although the GEA group exhibited numerically lower egg shedding than POS ($p = 0.08$) and the NEA ($p = 1.0$), these differences were not statistically significant. The median cumulative EpG values were 36,930 for POS, 620 for BVAX, 6,985 for GEA and 24,845 for NEA. The data on the egg shedding of the individual sheep is shown in Fig. 6.

In comparison to the POS, the highest worm burden reduction was observed in the BVAX (86.40%), whereas the GEA had a lower reduction (25.40%), and the NEA group showed no reduction. There were significant differences between BVAX and the GEA ($p = 0.04$), between the BVAX and the POS ($p < 0.01$) and BVAX and NEA group ($p = 0.01$). While the impact on the worm burden was not that pronounced, the number of eggs per female worm were significantly lower in GEA and BVAX groups compared to those of the POS group. There were differences between the POS and GEA ($p < 0.01$) and between the POS and the BVAX group ($p < 0.01$). GEA showed the overall lowest median value (7.98) (Fig. 7).

In addition to these parameters, a possible impact of the vaccine on the establishment rate of ingested larvae was investigated. NEA and GEA groups showed establishment rates of 48.44 and 41.90%, respectively, similar to or slightly below 44.86% observed in the POS group. However, none of these reached the low establishment rate seen in the BVAX group (10.28%).

The female: male ratio of recovered worms was 1.29 in the POS group (min of 0.78, max of 1.67), 0.59 in BVAX (min of 0.18, max of 1.02), 1.25 in GEA (min of 0.82, max of 2.10), and 1.24 in NEA (min of 0.94, max of 1.48). The number of eggs per female worm was lower in GEA and BVAX groups compared to those of the POS group ($p < 0.01$ for both comparisons) (Fig. 7).

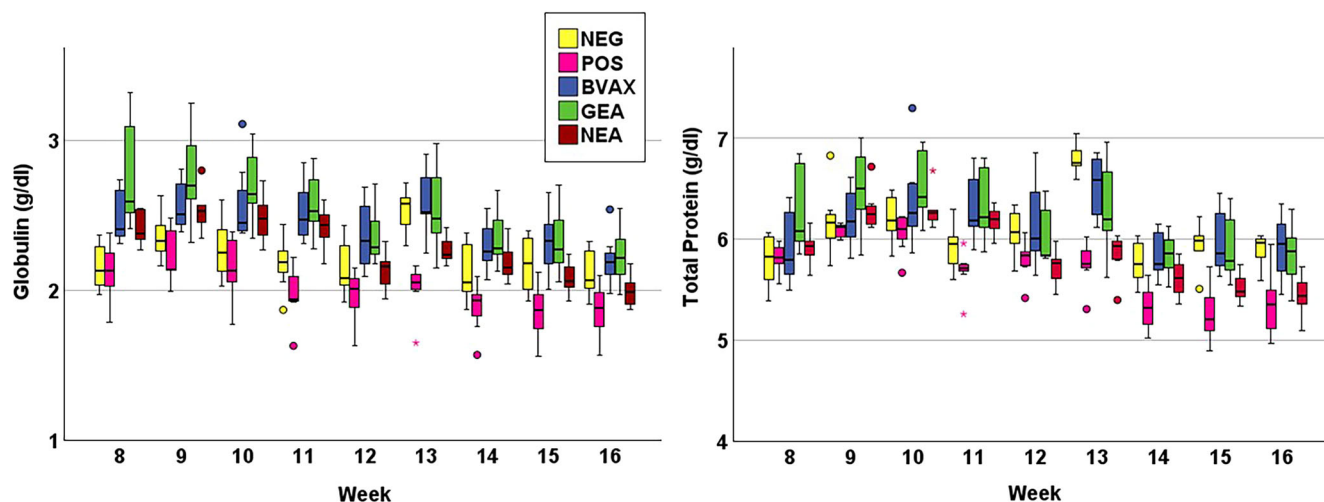


Fig. 4 | Globulin and total protein concentrations in serum in g/dl before ($<$ week 10) and after ($\geq$ week 10) challenge. Sheep in the POS (pink), BVAX (blue), GEA (green) and NEA (red) group were challenged with 5000 *H. contortus* L3 in week 10.

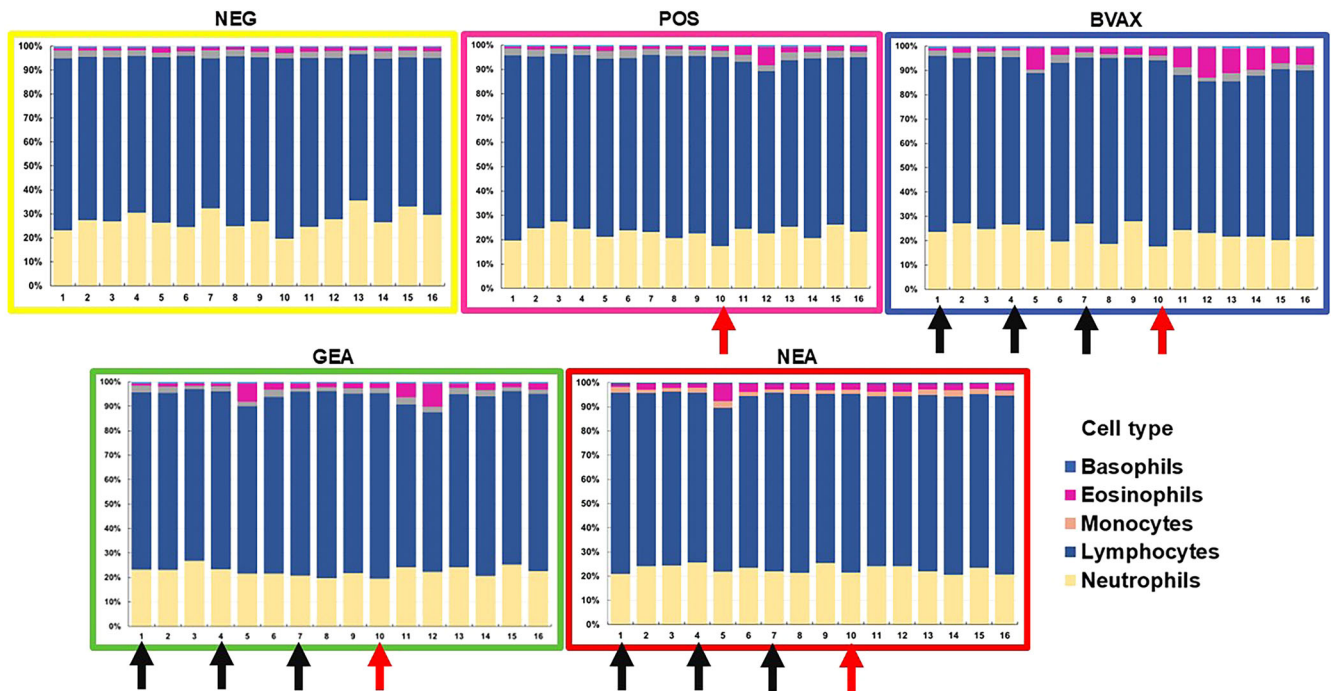


Fig. 5 | Relative distribution of granulocytes of the five groups (for color code details see caption Fig. 1). The black arrows highlight the 1st (week 1), 2nd (week 4) and 3rd (week 7) vaccination with either BVAX, GEA or NEA. The red arrows highlight the week when the sheep were challenged with 5000 L3 *H. contortus*.

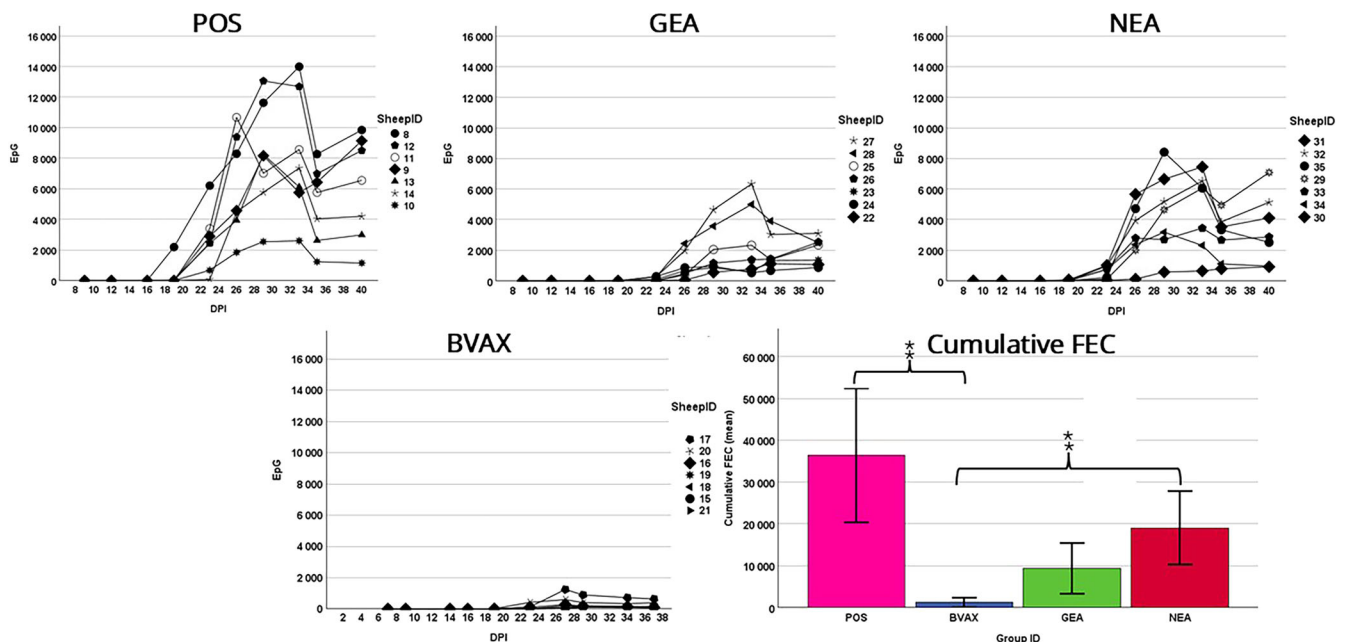


Fig. 6 | Individual fecal egg count (FEC; as eggs per gram of feces [EpG]) after challenge with 5000 *H. contortus* L3 (DPI: days post infection) of the POS, BVAX, GEA, and NEA group. The vaccinated sheep showed a delayed onset of egg

shedding in comparison to the POS. Egg shedding in the POS ranged from 10,005 to 60,425 EpG, in the BVAX from 340 to 3630 EpG, in the GEA from 3580 to 19,365 EpG and in the NEA from 3080 to 28,480 EpG. The error bars indicate the 95% CI.

Animals in the GEA-vaccinated group show a faster increase of parasite- and vaccine-specific serum immunoglobulins

To determine whether vaccination with the recombinant antigens GEA and NEA elicited specific antibodies in animals, sheep sera collected during the trial were examined by ELISA. ELISA data initially indicated that the total serum IgG and IgM antibodies did not change among the groups and during the trial (Fig. 8). Subsequently, serum samples were analyzed using ELISA

plates coated with either soluble proteins of adult *H. contortus* or with vaccine antigens (indirect ELISA). *Haemonchus*-specific IgG antibodies were induced in the vaccine groups (BVAX, GEA, NEA; Fig. 9A). Vaccination with GEA and NEA led to a faster increase of IgG levels compared to sheep in the BVAX group, which showed a more gradual antibody increase. Animals in the BVAX group maintained the highest IgG levels of all vaccine groups after the third vaccination, in line with the assumption that BVAX

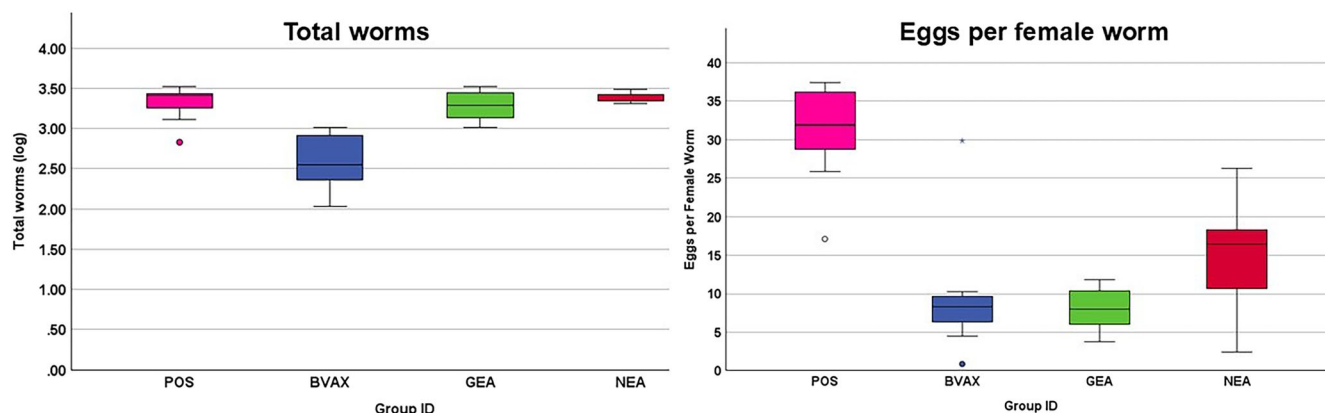


Fig. 7 | Number of eggs per female worm and worm burden. Left) Overview of the abomasal worm burden of 28 sheep euthanized 40 days after challenged with 5000 *H. contortus* L3. Right) Ratio of eggs per female in the different groups.

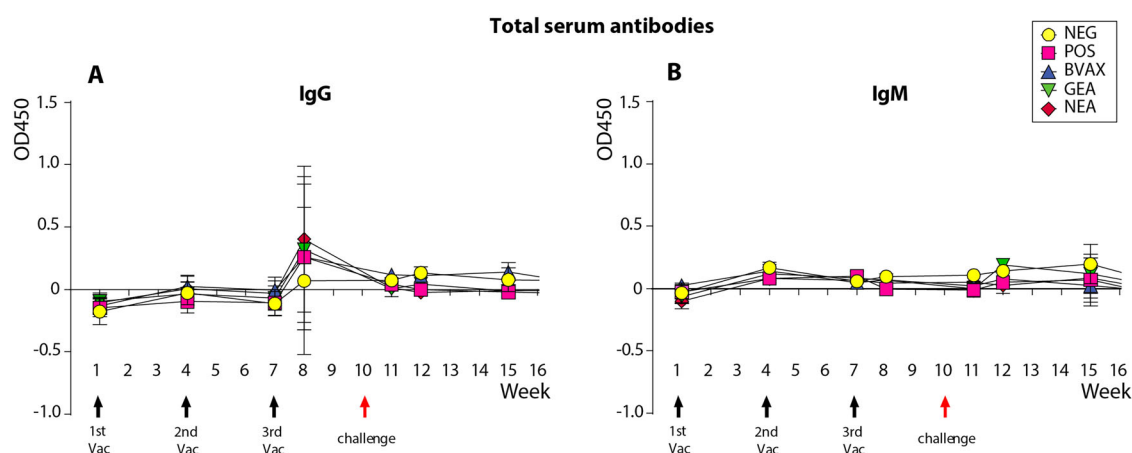


Fig. 8 | ELISA analysis of total serum antibodies in sheep. Panels show responses of baseline corrected-value total IgG (A) and IgM (B) antibodies, respectively. Assays were performed using plates coated with sheep sera.

contains more than the described antigens. As seen for other parameters, vaccination of animals with GEA led to a higher level of IgG antibodies compared to antibodies induced by NEA. This pattern was also observed for *Haemonchus*-specific IgE antibodies (Fig. 9B). No changes were detected for IgM antibodies in any group (Supplementary Fig. 3).

To assess the immune responses of individual animals, vaccine-specific antibodies were assessed using plates coated with either the corresponding vaccine antigens, whereas for the control groups (NEG and POS) Barbervax®-coated plates were used. The results indicated elevated levels of antigen-specific IgG (Fig. 9c) and IgE (Fig. 9d) antibodies in all three vaccine groups (BVAX, GEA, NEA). Although less pronounced in the BVAX group compared to GEA and NEA groups, IgG and IgE specific to the respective vaccine antigens were induced post vaccination but slowly tapering off during the trial. Analysis of IgA and IgM antibodies did not reveal differences between the vaccine groups and control group; vaccine-specific salivary IgA antibodies were also measured using antigen-coated plates. However, no obvious changes were observed (Supplementary Fig. 3).

Sera of GEA-vaccinated animals show a similar antigen-detection spectrum compared to sera of Barbervax®-vaccinated animals

In a further set of experiments, the antigen recognition patterns of GEA-induced IgGs were compared to those seen in sera of Barbervax®-induced IgG. The first experiment demonstrated that IgGs in sera of the BVAX and GEA groups recognized several protein targets with a broad molecular weight range (20–250 kDa) by Western-blotting. These proteins were not only

present in adult-stage parasite but also in the infective L3 larvae (Fig. 10a; Supplementary Figs. 4 and 5). This suggested a broader antibody targeting upon infection. *Haemonchus*-specific IgG antibodies in the sera of the GEA vaccinated group were detectable after the 1st vaccination (week 4), presumably a few weeks earlier than the appearance of IgGs in sera of sheep vaccinated with Barbervax® (BVAX group).

In addition to the appearance of IgGs, the binding spectrum of antibodies in animals vaccinated either with GEA or BVAX was compared. Antibodies in sera of animals vaccinated with GEA seemed to recognize many of the native antigens present in Barbervax® (Fig. 10b; Supplementary Figs. 6–8). Indeed, the IgG recognition patterns towards Barbervax® antigens displayed a comparable intensity and a similar molecular weight range. Similarly, sera from sheep vaccinated with Barbervax® showed comparable pattern recognition. Immunoblots were also performed on deglycosylated antigens, using Concanavalin A as a control to verify the completion of deglycosylation (Fig. 10c; Supplementary Figs. 9–11). IgG binding to the native antigens of Barbervax® was decreased after metaperiodate treatment in both groups, while the recognition of GEA by BVAX IgG was completely abolished, strongly suggesting a glycan-biased targeting manner. In contrast to Barbervax®-induced IgG in the BVAX group, the intensity of the GEA group IgG towards deglycosylated GEA did not change.

Pathohistological examination

The pathohistological examination showed a cell infiltration of the lamina propria mucosae of the sheep in the BVAX and the GEA group. One ileum and one abomasum sample are shown as a representative for respective

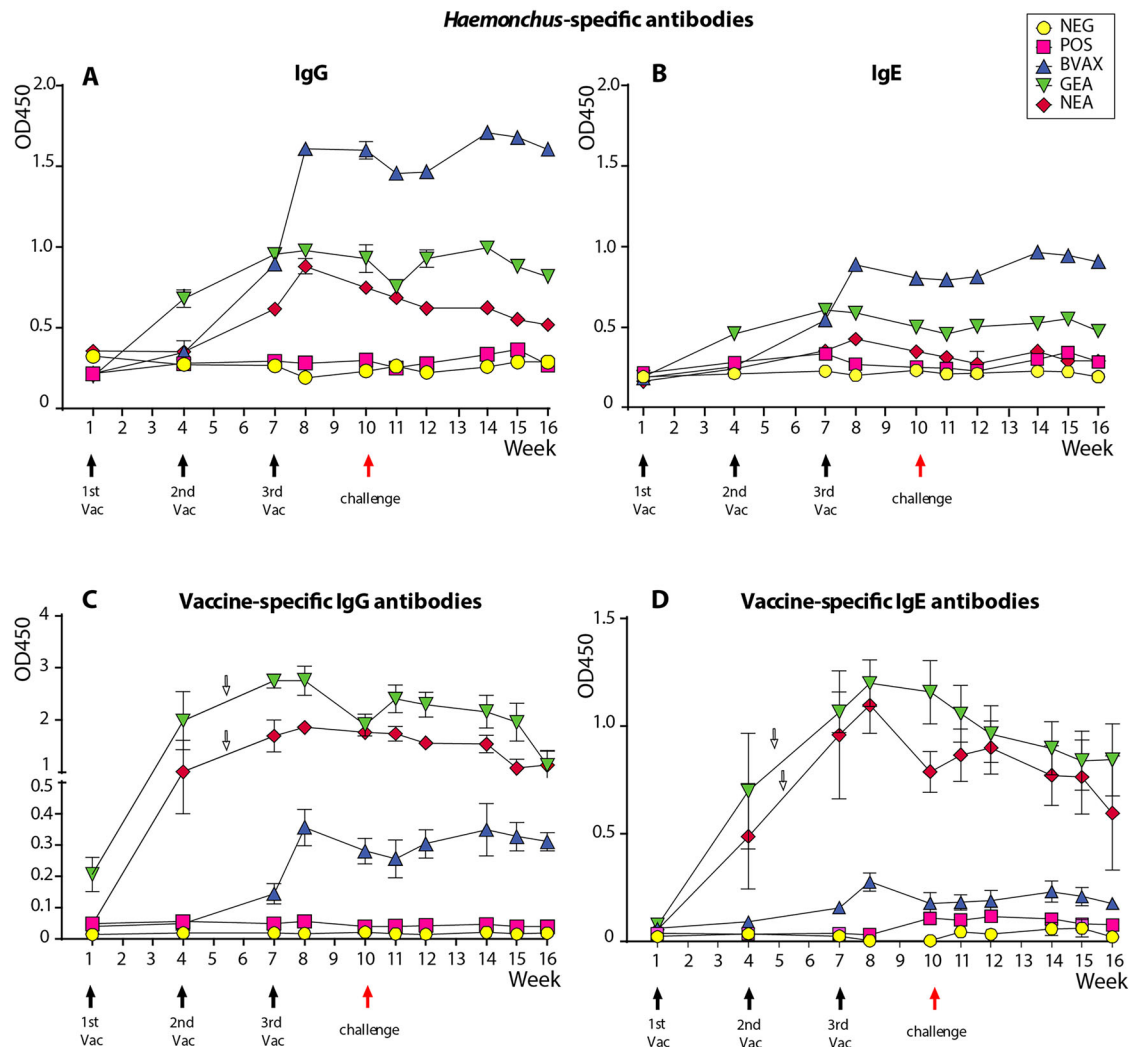


Fig. 9 | ELISA analysis of specific serum antibodies in sheep. A, B show responses of *Haemonchus*-specific IgG and IgE antibodies, respectively. Assays were performed using plates coated with soluble proteins of adult parasites and combined sheep sera. C, D illustrate antigen-specific IgG and IgE responses in individual sheep. Vaccine-specific antibodies for the GEA and NEA groups were measured using

plates coated with an equal amount of the corresponding vaccine antigens (marked with white arrows); the BVAX and negative and positive control groups were assayed using Barbevax[®]-coated plates. Data are plotted on different y-axis scales to better visualize the pattern of the different assays.

group (Supplementary Fig. 1; Supplementary Fig. 2). The ileum of the sheep from the BVAX group showed a higher cell density in the lamina propria mucosae in comparison to the other study groups.

Discussion

The hematophagous parasite *H. contortus* is the most pathogenic trichostrongylid in small ruminants and South American camelids. Current control strategies primarily rely on the application of anthelmintic drugs; however, the prevalence of anthelmintic resistance is emerging worldwide^{9–10,12}. Barbevax[®] has been the only commercialized vaccine against *H. contortus*, with high efficacy being reported in a number of trials in sheep, goats and alpacas^{18,29–31}. Despite this, Barbevax[®] has two main drawbacks: First, it remains inaccessible in many countries due to biosecurity concerns, despite the fact that a safe, well-defined, and effective recombinant vaccine is urgently needed. Second, the vaccine relies on a constant need to harvest antigen mixtures from worms retrieved from the stomachs of slaughtered sheep, as it is currently practiced, which makes it less attractive to ensure adherence to 3 R guidelines. Thus, a vaccine containing defined antigens that can be produced cost-effectively at large scale in fermenters under controlled conditions, ensuring high hygiene and biosecurity standards, is urgently needed and would pave the way for the

availability of vaccine doses year-round and worldwide. Suitable antigens have been indicated in studies using H11 and H-gal-GP antigens of the commercial vaccine Barbevax[®]. These studies further showed that these antigens are naturally glycosylated, carrying very distinct N-glycan structures compared to those found in mammals^{32,33}. This glycosylation has made the production of recombinant forms of such antigens extremely challenging.

Previous studies using a combination of two recombinant H11 isoforms (antigen expressed in *C. elegans*; three injections with a saponin-based adjuvant; challenge with 5000 L3) reported no significant difference in fecal egg shedding, worm burden or worm female: male ratio in comparison to the adjuvant control group in Suffolk-cross lambs²⁵. While this study showed promising methodological results, it also highlighted that correct glycosylation may play a far more important role than anticipated. Thus, the safety and efficacy of two recombinant vaccines produced in Hi5 insect cells, one in a glycoengineered form (GEA), the other in non-glycoengineered form (NEA), were evaluated in a randomized controlled vaccine trial in the present study. To the authors' best knowledge, the presented animal trial is the first successful attempt to vaccinate sheep with an antigen cocktail consisting of glycoengineered recombinant proteins produced in insect cells against a parasitic worm. Overall, both GEA and NEA did not show any

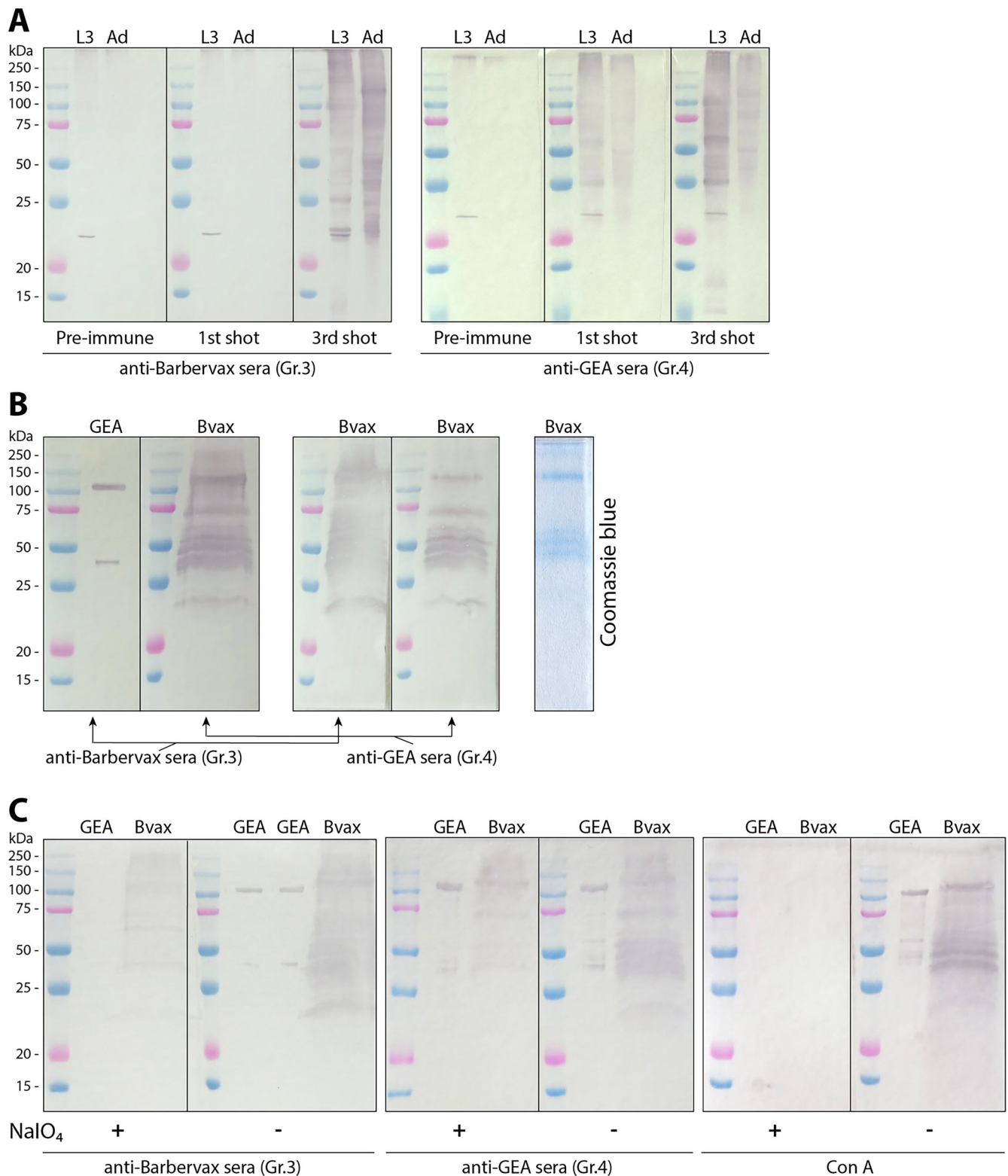


Fig. 10 | Recognition patterns of serum IgG antibodies to native and recombinant *H. contortus* antigens in vaccinated sheep. A vaccination with either Barbervax[®] (BVAX) or GEA elevated IgG antibodies that bound to soluble proteins of L3 larvae (L3) and adult-stage (Ad) parasites. Parasite-specific IgG appeared earlier in GEA group than in BVAX group. **B** Cross-reactivities of IgG were observed in both

BVAX- and GEA-antisera; the comparison of Barbervax[®]-specific IgG (right panel) between the two groups suggested a similar binding pattern and intensity.

C Deglycosylation of antigens using sodium metaperiodate (NaIO₄) reduced Barbervax[®]-specific IgG targeting in both groups and completely abolished the recognition of GEA by BVAX IgG.

appreciable negative side-effects in vaccinated animals. Both vaccines showed significant impact on the parasite, with egg shedding in the GEA group was reduced by 81.09% and worm burden decreased by 25.36%. A minimum efficacy of > 65% is required to have a positive epidemiological

effect to control haemonchosis (e.g., reduced pasture contamination with subsequently lower worm burdens)³⁴, and the presented GEA vaccine fulfilled this requirement in the present experimental trial with a wide margin. Despite a reduction of egg shedding by 32.72% in the NEA group compared

to the unvaccinated group, no significant reduction in the worm burden was observed in this group. The comparison between GEA and NEA groups strongly indicated that glycoengineering is essential for an improved immune response against *H. contortus* since the GEA group continuously showed a more favorable outcome in parasitological and hematological parameters compared to the NEA group. Our data therefore confirm that nematode-type glycan modifications are crucial in the production of an anti-*Haemonchus* vaccine. It needs to be highlighted that the statistical difference and the biological relevance of the findings in this study need to be considered and will require further investigation. For example, the significantly increased lymphocyte concentration in the GEA group in comparison to other groups needs to be further investigated.

According to the manufacturer's instructions for Barbervax®, the local tissue reactions in the form of swelling at the injection site can last for up to 17 days³⁵. In the present study, the injection site was assessed on daily basis for seven days after each vaccination. There was no difference between groups apart from a transient increase of body temperature following the second vaccination, which suggests that the insect cell-derived antigens are clinically safe. *Haemonchus contortus* is hematophagous and can cause severe anemia in hosts³⁶. As anemia in infected sheep is often accompanied by nutrient loss, either directly by the worms or indirectly due to the damaged intestinal epithelial layers, weight loss/regain after challenge was compared. Before challenge, there were no differences in daily weight gain between the groups, while after challenge the BVAX group showed the lowest body weight gain, which was somewhat unexpected given that BVAX showed the highest reduction in worm burden/egg counts. This phenomenon can currently not be explained. Many sheep are kept for meat production, with body weight gain being a key performance indicator to measure the economic benefit of the farm. Thus, the differences in daily body weight gain suggest that is prudent to suggest that this parameter will be included in future vaccine trials as an important criterion. Even though individual feed intake was not measured, one can speculate that either daily dry matter intake was lower in the BVAX group (e.g., due to reduced appetite, abdominal pain) or that other mechanisms, such as an excessive immune reaction, caused the reduced daily weight gain. Indeed, vaccination against intestinal nematodes causes granulocyte infiltration in the mucosal layers. In previous works, during infection with trichostrongyloids a potent chemoattractant activity for ovine bone marrow-derived eosinophils was noted in vitro³⁷. This activity was identified as a chemoattractant protein in whole worm extracts of third and fourth larval and adult stages of *Teladorsagia circumcincta* as well as adult stages of *H. contortus*. The resulting local eosinophil-mediated mucosal damage, comparable to that seen in asthmatic lungs, may provide a permissive local micro-environment for the parasite for increased attachment and feeding^{37,38}. Overall, eosinophils have been reported to play a significant role in gastrointestinal nematode infection in sheep³⁹. A negative association between blood eosinophils and FEC was observed in *H. contortus* infections⁴⁰, and in vitro assays demonstrated that eosinophils may kill *H. contortus* larvae, thereby reducing parasite establishment⁴¹.

In the present study, pathohistological examination of the ileum of sheep in the BVAX group showed a higher density of cells in the lamina propria mucosae, compared to the other groups, which underscores this assumption. Additionally, the number of circulating eosinophils after challenge was higher in the BVAX group. It is therefore possible to speculate that the heightened immune response in the BVAX group, represented by higher concentrations of peripheral eosinophils and a higher cell density in the ileum, contributed to the reduced body weight gain. Furthermore, higher concentrations of eosinophils in the blood of sheep vaccinated with GEA compared to the blood of sheep vaccinated with NEA was observed. Following the 3rd vaccination the eosinophils did not increase as drastically in all three experimental groups compared to the increase seen after the 1st and the 2nd injection. While pre-activation of eosinophils by vaccination may be an essential part of the induction of a protective immune response, subsequently limiting worm establishment, this assumption needs to be

corroborated by analyzing the cytokine and chemokine profile in sheep sera generated from the different groups.

In addition to eosinophil concentration in peripheral blood, we also analyzed further blood parameters related to worm infection. Packed cell volume and hemoglobin levels can decrease severely after *H. contortus* infection since the sheep may lose up to 0.2 to 0.6 liters of blood per day⁴². In the present study, sheep vaccinated with GEA showed a higher total protein (5.95 versus 5.58 g/dl) and packed cell volume (26.77 versus 26.11%) compared to sheep vaccinated with NEA. These results indicate a further benefit of GEA that seems to notably reduce blood losses through worm feeding. Even though the reduction in the worm burden in sheep vaccinated with GEA was only 25.36%, the PCV and Hb levels indicate that the remaining worms in GEA vaccinated animals were probably weakened. Whether this is part due to the impact of GEA on the integrity of the female worms, supported by the reduced number of female eggs per worm, remains to be followed up in detail in further trials. In this context, we also established whether the vaccine responses depended purely on secretory IgA, or whether a systemic adaptive immune response was involved. Our data suggested that IgG and IgE were the major isotypes that were elevated post-vaccination, post-infection, as well as post-challenges. There was a link between parasite-specific antibodies and efficacy, with higher IgG/IgE level being associated with lower EpG values and worm burdens. The overall antibody titers specific for *Haemonchus* in the GEA group were lower than those in the BVAX group over the trial period, which could be the cause of a lower efficacy in this group.

The low titer of vaccine-specific antibodies observed in the BVAX group might be due to the low antigen coating efficiency despite optimization efforts. Independent of potential technical issues, ELISA data suggested that antibody in sera of sheep in the GEA group and NEA group increased quickly in comparison to the BVAX group, which indicated that the recombinant *H. contortus* antigens in the GEA and NEA preparation may be more efficient in inducing antigen-specific antibody responses compared to those being in the native worm antigens in Barbervax®.

Western blot analysis confirmed this observation and further revealed an unexpectedly broad antibody recognition pattern where numerous proteins from both L3 larvae and adult parasites were recognized by vaccine-induced antisera. Notably, IgG antibodies in sera of Barbervax®-vaccinated animals exhibited a strong bias toward glycan moieties, which is in line with literature²⁵. Similarly, anti-glycan IgG antibodies were detected in animals from the GEA group, alongside the ones specific to protein backbones. These findings implicate that the vaccine antigens, both the native and glycoengineered forms, can efficiently trigger antibody production in sheep that target a much broader range of glycoproteins than previously thought.

The novel glycoengineered vaccine GEA, comprising five glycoengineered recombinant antigens was tested for the first time in vivo in sheep and demonstrated that glycoengineering is essential to achieve a significant reduction in egg shedding and worm burden, and to improve hematological parameters. Although the overall efficacy of GEA at 100 µg/dose was lower than the commercial vaccine Barbervax®, the efficacy benchmark in this trial, animals in the GEA group exhibited greater body weight gain following challenge infection. Direct comparison, however, is complicated by the fact that Barbervax® contains native H11 and additional components of the H-gal-GP complex, whereas GEA consists of a defined cocktail of glycoengineered recombinant H11 isoforms. However, it opens the way for future development of highly effective recombinant vaccines against this and other parasitic worms. To enhance the effectiveness of GEA, further studies to investigate the most effective recombinant antigen isoforms, the vaccine dose as well as immunization schedules are needed. While we are aware that the small sample size of our study has its limitations, we still obtained some interesting and promising results. It is clear, though, that future trials should not only focus on vaccine formulations and adjuvant evaluation, but also include larger, more physiological and genetically diverse sample groups to improve the general applicability of the findings. Despite this, our study demonstrates the promising potential of glycoengineered recombinant

antigens as effective substitutes for native worm-derived components in helminth vaccine development. The glycoengineering approach offers precise antigen composition, enhanced safety and scalable production, addressing key challenges in vaccine manufacturing. Furthermore, this strategy lays a valuable foundation for advancing helminth vaccine research and addressing the growing issue of AR. In conclusion, these findings mark a step toward the development of recombinant helminth vaccines, contributing to improved control of parasitic diseases.

Methods

Ethical considerations

The study 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 guidelines⁴³.

Sample size calculation and randomization

The sample size calculation was based on the following assumptions: vaccine efficacy is assessed by evaluating the number of excreted eggs (eggs per gram (EpG) of feces; EpG) and the worm burden (number of worms in the abomasum). In the work by González-Sánchez et al.⁴⁴) (publication Table 2), the standard deviation was approximately half of the mean of the total egg excretion, and the difference between the vaccinated and non-vaccinated groups was about 75%⁴⁴. With a significance level of 5%, a power of 80%, and using a one-sided test procedure (vaccinated animals excrete fewer eggs per gram of feces), the calculated group size was seven animals per group. The sheep were allocated to each study group randomly (stratified randomization) depending on their body weight and breed using the “rand()” function in Microsoft Excel.

Sheep

The sheep were born between August and October 2023 (average weight of 26.7 kg; average age of 4 months) and raised on the same dairy sheep farm in the federal state of Lower Austria. The farm is run as a commercial organic dairy sheep farm with ~200 dairy sheep. The ewes were thoroughbred Lacaune and the three rams were thoroughbred Lacaune or Jura. Therefore, the lambs were Lacaune x Lacaune or Lacaune x Jura breed. The lambs were born and remained with their mothers for 14 days before being moved to a separate barn. The lambs did not have any access to pasture, only to a concrete outdoor loafing area. The farmer separated 39 male lambs for the

animal trial and housed them according to the national regulations. All lambs were vaccinated against clostridial disease on the farm within the regular herd health management procedure using Miloxan® (Boehringer Ingelheim Animal Health France SCS, Lyon, France) with a dosage of 2 ml

Table 3 | Differences in the differential blood counts between the five groups

Group ID	Mean [95%CI]	Overall p value
Lymphocytes /μl		
NEG ^a	4144.92 [3892.84; 4396.99]	< 0.01
POS ^{b**}	3890.61 [3638.54; 4142.68]	
BVAX	4391.28 [4139.21; 4643.35]	
GEA ^{a*,b**,c*}	4679.40 [4427.33; 4931.48]	
NEA ^{c*}	4004.36 [3752.28; 4256.43]	
Monocytes /μl		
NEG	167.00 [139.39; 194.61]	0.08
POS	143.44 [115.83; 171.05]	
BVAX	165.81 [138.21; 193.42]	
GEA	148.53 [120.92; 176.14]	
NEA	116.57 [88.96; 144.18]	
Eosinophil Granulocytes /μl		
NEG ^{a**}	97.38 [28.44; 166.33]	< 0.01
POS ^{b**}	138.45 [69.50; 207.40]	
BVAX ^{a**,b**,c**,d**}	507.17 [438.22; 576.11]	
GEA ^{c**}	188.82 [119.87; 257.76]	
NEA ^{d**}	143.78 [74.83; 212.72]	
Basophil Granulocytes /μl		
NEG ^{a**}	36.33 [31.22; 41.43]	< 0.01
POS ^{b**}	36.96 [31.85; 42.06]	
BVAX ^{a**,b**,c**}	52.75 [47.65; 57.86]	
GEA	42.82 [37.71; 47.93]	
NEA ^{c**}	33.78 [28.67; 38.88]	
Segmented Granulocytes /μl		
NEG ^{a**,b**,c**,d**}	1874.23 [1755.93; 1992.52]	< 0.01
POS ^{a**}	1292.75 [1174.46; 1411.05]	
BVAX ^{b**}	1421.45 [1303.16; 1539.75]	
GEA ^{c**,e*}	1514.37 [1396.07; 1632.66]	
NEA ^{d**,e*}	1233.35 [1115.05; 1351.64]	

Mean values and 95% confidence intervals are shown, the significance level was set at $p \leq 0.05$. All superscript letters highlighted by * indicate p values ≤ 0.05 . Superscript letters with ** indicate p values < 0.01 . The p values in the right column indicate differences between the groups overall.

Table 2 | Differences in total protein and albumin between the five groups after challenge in week 10

Group ID	Mean [95% CI]	Overall p value
Total Protein (g/dl)		
NEG ^{a*,b*}	5.97 [5.81; 6.14]	< 0.01
POS ^{a*,c*,d*}	5.58 [5.41; 5.74]	
BVAX ^{c*,e*}	6.05 [5.89; 6.21]	
GEA ^{d*,f*}	5.95 [5.78; 6.11]	
NEA ^{b*,e*,f*}	5.58 [5.42; 5.75]	
Albumin (g/dl)		
NEG ^{a**}	3.83 [3.73; 3.93]	< 0.01
POS	3.66 [3.56; 3.76]	
BVAX ^{b*}	3.76 [3.66; 3.86]	
GEA	3.65 [3.54; 3.75]	
NEA ^{a**,b*}	3.52 [3.42; 3.62]	

All superscript letters highlighted by * indicate p values ≤ 0.05 . Superscript letters with ** indicate p values < 0.01 . The p values in the right column indicate differences between the groups overall.

Table 4 | Mean body temperature and standard deviations in degree Celsius (°C) after vaccination with BVAX, GEA and NEA

Vaccination	BVAX	GEA	NEA	p value
1 (day 0)	39.86 ± 0.19	40.04 ± 0.46	39.93 ± 0.30	0.59
1 (day 1)	39.64 ± 0.32	39.86 ± 0.35	39.76 ± 0.20	0.42
2 (day 0)	39.54 ± 0.73	39.87 ± 0.57	40.36 ± 0.60	0.08
2 (day 1)	39.33 ± 0.27	40.20 ± 0.66	39.80 ± 0.24	0.01*
3 (day 0)	39.03 ± 0.32	39.00 ± 0.17	39.10 ± 0.26	0.76
3 (day 1)	39.45 ± 0.49	39.09 ± 0.52	39.33 ± 0.27	0.30

Body temperature was measured on the day of vaccination (day 0) and one day after the vaccination. P values show the results of the one-way ANOVA. * Highlights significant values.

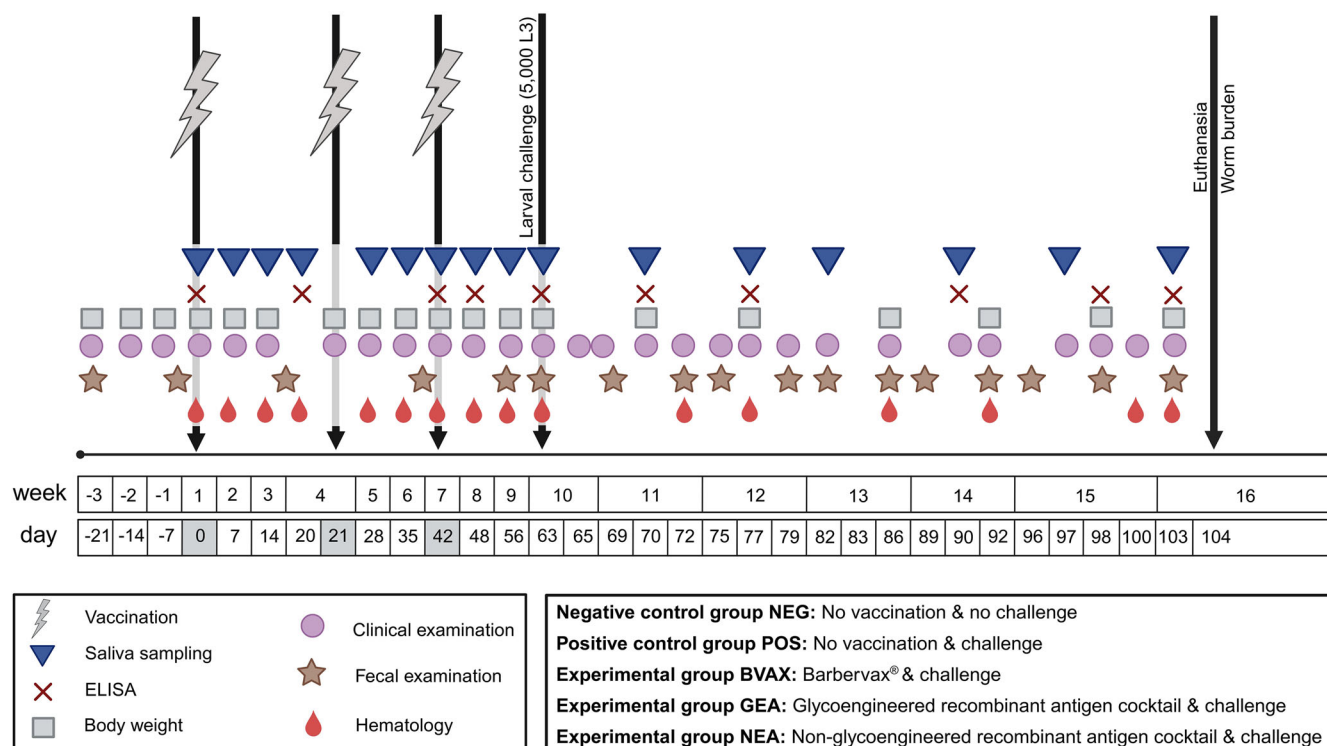


Fig. 11 | Overview on the specimen material collected during the vaccine trial from Jan to May 2024. The sheep were kept in five groups with seven sheep in each group. The negative control group (NEG) received no vaccination and no challenge. The positive control group (POS) and the experimental groups were challenged with 5000 *H. contortus* L3 (in week 10). The experimental groups were vaccinated three times

subcutaneously with Barbervax® (BVAX), the novel glycoengineered vaccine (GEA) or the non-glycoengineered vaccine (NEA). Examinations, sampling dates and ELISA test dates are shown [Created in BioRender. Sajovitz-Grohmann, F. (2025) <https://BioRender.com/pkpsk5>].

per sheep subcutaneously. They received the first immunization on October 25th, 2023, and the second one on November 28th, 2023.

Deworming strategy before the trial

The first deworming was carried out on December 15th, 2023, where the sheep received 0.2 mg/kg body weight (BW) of ivermectin (Ivomec® 10 mg/ml, Boehringer Ingelheim Animal Health) subcutaneously. At the farm of origin, the sheep were assessed, and the heaviest sheep was weighed and the dosage was chosen after that. Additionally, fecal samples were collected in September 2023 and examined for gastrointestinal nematodes, with negative results. At the day of arrival (January 2nd and 3rd, 2024) the sheep were treated orally with 5 mg/kg BW of fenbendazole (Panacur® 250 mg, Intervet GesmbH, Vienna), 7.5 mg/kg BW of netobimin (Hapadex® 50 mg/kg, Intervet GesmbH, Vienna) and 20 mg/kg BW of toltrazuril (Baycox® Multi 50 mg/ml, Bayer Animal Health GmbH, Leverkusen, Germany).

Vaccine efficacy trial in vivo

The randomized controlled vaccine trial was carried out at the Clinical Center for Ruminant and Camelid Medicine, University of Veterinary Medicine, Vienna, Austria. The sheep were housed in a separate building and divided into five groups of seven animals (Fig. 11). All groups received the same amount of hay (not weighed) twice daily and water was provided *ad libitum*. In total, four groups were challenged with 5,000 in-house produced *H. contortus* L3 larvae, an isolate from Boehringer Ingelheim Vet-medica GmbH, Kathrinenhof Research Center, Rohrdorf, Bavaria, Germany. A negative control group (NEG; no vaccination and no challenge) and a positive control group (POS; no vaccination and challenge) were included. The seven sheep in each experimental group were vaccinated three times subcutaneously either with 1 ml Barbervax® (BVAX; Wormvax Australia Pty Ltd., Albany, WA, Australia; imported from UK via Merlin

Vet, Kelso, UK), or with 1 ml of the glycoengineered recombinant antigen cocktail (GEA,²⁸) or with 1 ml of the non-glycoengineered recombinant antigen cocktail (NEA). Recombinant vaccines (GEA and NEA) were formulated by mixing 100 µg of insect cell (Hi5)-derived recombinant products (H11, H11-1, H11-2, H11-4 and GA1; 20 µg/antigen/dose) with 1 mg of Quil-A® adjuvant (InvivoGen, San Diego, CA, USA) in PBS solution. Sheep were challenged three weeks after the third vaccination using a 2 ml disposable plastic pipette (dosage 2 ml). Multiple samples, including fecal, blood and saliva samples, were collected from animals during the study period (Fig. 11). The sheep were held by one person and euthanized by a veterinarian using an intravenous dosage of 60 mg/kg BW of Pentobarbital-Natrium (Release®, 300 mg/ml, WDT, Garbsen, Germany). Auscultation of the heart and the lungs, and subsequently testing the absence of the cranial reflexes, was performed to verify the dead.

The production of the glycoengineered recombinant antigen cocktail is patented under the intellectual property IP (European Patent Application No. EP24216456.4 entitled “Expression system for glycoengineered antigens” filed November 29, 2024). Detailed description of the production and in vitro characterization of the antigen cocktail is described by Adduci et al., 2025²⁸.

Clinical assessment and differential blood counts

The clinical assessment (e.g., body temperature, FAMACHA®) was carried out using a standardized protocol for physical examination^{45–47}. Blood samples (EDTA, serum, Tempus™ Blood RNA Tubes [Thermo Fisher Scientific, Waltham, MA, USA]) were drawn from the external jugular vein at defined time points throughout the study period (Fig. 11). The sheep were weighed using Gallagher W0 digital scales (Gallagher Europe, Groningen, The Netherlands). Hematological parameters of whole blood sample, including leukocytes, erythrocytes, hemoglobin, and erythrocyte indices, were analyzed by flow cytometry (Advia® 2120i, Siemens, Erlangen, Germany). Total protein and albumin were measured by photometry (Cobas®

pure C303, Roche Diagnostics International Ltd, Rotkreuz, Switzerland). During the whole study period, the vaccination was carried out after the clinical assessment and blood sample collection.

Assessment after vaccination and local injection site reaction

The 21 sheep from the BVAX, GEA and NEA groups underwent eight assessments following each vaccination. The local injection site was assessed on the day of vaccination (morning: vaccination; afternoon: first assessment), and then once daily until day 7 after vaccination, following the protocol from Stadler and coworkers, which was adapted for sheep⁴⁸. The following signs of the injection site and the surrounding area were assessed: swelling (none; slight; severe), pain during palpation (yes/no), necrosis (none; slight; severe), tissue induration (none; firm; hard) and lymph node enlargement (none; slight; severe).

Preparation of soluble *H. contortus* proteins

Soluble proteins from adult *H. contortus* were extracted using a two-step homogenization process. Briefly, adult worms were resuspended in Tris-buffered saline (100 mM Tris-HCl, 100 mM NaCl, pH 7.4) supplemented with 0.02% sodium azide, 1% octylthioglucoside, and a 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). The worms were first homogenized on ice for 2 min with intermittent pauses using a Turrax® dispersion tool (T10 basic, equipped with an S10N-10G-ST probe; IKA, Staufen, Germany). The homogenate was then transferred to an ice-cold Dounce homogenizer for manual homogenization. Resulted homogenate was centrifuged at 12,000 × g for 30 min at 4 °C. The supernatant was filtered through 0.22 µm sterile syringe filters and collected into sterile Falcon tubes. For larval (L3) parasites, due to the limited material, homogenization was performed in 1.5 ml tubes using disposable polypropylene pestles (Thermo Fisher Scientific). Protein concentration was measured using a Pierce™ Bradford Plus protein assay kit (Thermo Fisher Scientific).

Enzyme-linked immunosorbent assay (ELISA)

Native antigens of adult *H. contortus* and recombinant antigen cocktails (GEA and NEA) were diluted to a concentration of 2 µg/ml in coating buffer (200 mM carbonate/bicarbonate, pH 9.6). 96-well Nunc-Immuno™ MicroWell™ ELISA plates (Sigma-Aldrich) were coated overnight at 4 °C with 50 µl of antigens (100 ng/well). Post blocking with 2% BSA, sheep sera (dilution 1:100) were added to the wells and incubated at 37 °C for 1 h. Serum antibodies were probed with anti-sheep IgG (Sigma-Aldrich, dilution 1:10,000), IgE (Bio-Rad Laboratories Ges.m.b.H., Hercules, CA, USA; 1E7, dilution 1:10,000), IgM (Sigma-Aldrich; rabbit polyclonal, dilution 1:10,000) and IgA (Bio-Rad Laboratories Ges.m.b.H.; rabbit polyclonal, dilution 1:1,000). Horseradish peroxidase (HRP)-conjugated secondary antibodies, anti-mouse IgG (Sigma-Aldrich, dilution 1:5,000) and anti-rabbit IgG (Sigma-Aldrich dilution 1:10,000), were used to react with tetramethylbenzidine-hydrogen peroxide (Thermo Fisher Scientific). Post addition of a reaction stopping buffer (Carl Roth GmbH + Co. KG, Karlsruhe, Germany; 0.5 M sulfuric acid), optical density at 450 nm (OD450) was measured using a microplate reader (FilterMax F5; Molecular Devices, San Jose, CA, USA). Total serum IgG and IgM were examined by performing direct ELISA using an anti-sheep antibody (stated above) to probe sheep sera (1:100 diluted) coated into ELISA plates.

Western blotting and metaperiodate treatment

Worm-derived and recombinant antigens were dissolved in SDS-loading dye, denatured at 95 °C and loaded on 12% SDS-PAGE gels. Precision Plus Protein™ Dual Color Standards (Bio-Rad Laboratories GesmbH) was used as protein ladder. Post electrophoresis at 200 V, proteins were transferred onto nitrocellulose membranes using a TransBlot™ Turbo™ Transfer System (Bio-Rad Laboratories GesmbH). After blocking in 0.5% BSA, nitrocellulose membranes were incubated with sheep antisera, combined from the 7 sheep of the same group (dilution 1:200), then with a mouse anti-goat/sheep IgG (Sigma-Aldrich; dilution 1:10,000), and finally with an alkaline phosphatase (AP)-conjugated anti-mouse IgG (Sigma-Aldrich; Fc-specific; dilution

1:10,000). SIGMAFAST™ BCIP®/NBT (Sigma Aldrich) was used as the substrate for color development.

Protein deglycosylation was performed on-membrane using a protocol adapted from Roberts et al.²⁵. Briefly, immunoblots were treated overnight at 4 °C with 10 mM sodium meta-periodate dissolved in 0.05 M sodium acetate, pH 4.5, 0.05 M sodium chloride buffer, prior to subsequent blocking and incubation steps. To monitor the deglycosylation efficiency, a treated blot was incubated with biotinylated Concanavalin A (Vector Laboratories, Newark, CA, USA; Conn A, dilution 1:250) in PBS buffer supplemented with 1 mM calcium chloride followed by a goat anti-biotin secondary antibody (Sigma-Aldrich; dilution 1:10,000).

Mini-FLOTAC method

Feces were sampled and analyzed individually by Mini-FLOTAC with a detection limit of five eggs per gram (EpG)^{49,50}. Samples were weighed (5 g feces) and mixed with 45 ml of a saturated saline solution (density 1.18 g/ml) and sieved through a mesh (average mesh size: 1.3 mm) in a beaker afterwards. An equal distribution of the eggs was ensured by using a magnetic stirrer (IKA-COMBIMAG REO, Janke & Kunkel GmbH u. Co. KG, Staufen, Germany). Samples were quantitatively evaluated under the light microscope (Eclipse Ci-S, 100 x magnification, Nikon, Vienna, Austria). The EpG was calculated by multiplying the number of eggs counted in both chambers by 5 (multiplication factor for livestock feces)⁵⁰.

Worm burden and worm sexing

The worm burden was evaluated after euthanizing the sheep. First, the abdominal cavity was opened, and the duodenum was ligated. The omasum was cut away to keep the content within the abomasum. Subsequently, the abomasum was removed from the gastro-intestinal bundle and put into a bucket. After opening the greater curvature, the content was transferred into a sieve with a mesh width of 150 µm and washed thoroughly. Subsequently, the entire abomasal content was transferred into a bucket for storage and further assessment. The abomasal mucosa as well as the entire abomasal content were inspected for adult worms under good light conditions. All worms were collected, separated by gender and counted within three days after sacrifice^{51,52}.

Patho-histological examination

Abomasal and ileum tissues were collected for pathohistological examination *postmortem*. Briefly, abomasal tissue (location fundus region) and ileum tissue, in size of one square-centimeter, were taken immediately after opening the abdominal cavity using a scissor and forceps and fixed in 10% formalin (BiopSafe®, Vedboek, Denmark) in a 20 ml container. Samples were collected from three sheep from each group. Subsequently, the samples were dehydrated in graded series of ethanol and embedded in paraffin wax. Thereafter, approximately 3 µm thick sections were stained with HE for further assessment.

Statistics

Descriptive and explorative statistical analysis was performed using Microsoft Excel 2010 (Microsoft, Washington, USA) and IMB SPSS® Statistics Version 29 (IBM, New York, USA). Descriptive statistics were carried out using mean, median, 25 and 75th percentile, minimum and maximum. An extreme outlier was defined as: >75th percentile plus 3 times the inter-quartile range (IQR). Extreme outliers are shown as asterisks in the boxplots and normal outliers are shown as circles. Normal distribution was tested using the Kolmogorov-Smirnov test. The daily weight gain in gram was calculated before (63 days) and after (40 days) challenge by using the body weight at the begin of the trial (2nd/3rd January), before challenge (27th March) and at the end of the trial (6th May). For the packed cell volume, thresholds were calculated using the 25, 50, and 75th percentile. The cumulative egg excretion was calculated by summarizing the egg excretions over time (syn. cumulative FEC). The EpG values per worm was calculated by dividing the cumulative EpG values by the number of female worms (no normal distribution). The female/male ratio was calculated by dividing

female by male worms. The EpG values and worm counts were log transformed using the lg10 function in SPSS. All measures (EpG values, worm counts, blood values) were analyzed using a linear mixed model followed by a post hoc test (Sidak test) between the experimental groups. Group identification and sampling date were included as fixed values. Sampling date was included as repeated measure and sheep identification as subject. All missing values were excluded in the analysis. The Akaike's information criterion (AIC) revealed 215.24 for the egg excretion. Comparison between groups without repeated measures was carried out using the Kruskal Wallis test (> 2 samples) including the Bonferroni correction (e.g., worm burden, cumulative EpG), the Mann-Whitney U test (2 samples) or the Fishers exact test (< 5 cases). For comparison between groups with a normal distribution a one-way ANOVA was carried out. For all tests the significance level was set at $p \leq 0.05$.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

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Author contributions

S.Y., K.L., A.J., T.W., and D.W. conceived the project. S.Y., K.L., A.J., T.W., F.S.G., I.A., B.H., and D.W. contributed to the design of the methodology. S.Y., K.L., F.S.G., I.A., D.W., and A.T. carried out the formal analysis. S.Y., K.L., F.S.G., I.A., J.E., J.Z., B.P., L.W., S.W., and B.H. performed the experiments and collected the data. A.J., T.W., B.H., S.Y., K.L., F.S.G., I.A., S.W., and L.W. provided the resources in terms of labor resources, materials, reagents and analysis tools. F.S.G. and K.L. prepared the initial draft of the manuscript. F.S.G., K.L., and I.A. visualized the data. K.L., S.Y., T.W., A.J., B.H., and D.W. acted as supervisors. S.Y., K.L., F.S.G., and I.A. managed the project administration. S.Y., K.L., T.W., and A.J. acquired the funding. F.S.G., K.L., S.Y., A.J., D.W., T.W., I.A., B.H., L.W., B.P., S.W., J.Z., J.E., and A.T. reviewed and edited the manuscript and accepted the final version of the manuscript.

Competing interests

A patent application related to this work has been filed at the European Patent Office (application No. EP24216456.4). All other authors declare no other competing interests.

Additional information

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