Veterinary Parasitology 229 (2016) 15–21

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

# Vaccination of goats against *Haemonchus contortus* with the gut membrane proteins H11/H-gal-GP

Lorena Meier[a], Paul R. Torgerson[b], Hubertus Hertzberg[a,*]

[a] Institute of Parasitology, University of Zurich, Winterthurerstrasse 266a, 8057 Zurich, Switzerland
[b] Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 270, 8057 Zurich, Switzerland

ARTICLE INFO

*Article history:*
Received 20 March 2016
Received in revised form 23 August 2016
Accepted 31 August 2016

*Keywords:*
Goats
Haemonchus contortus
Control
Vaccination
Field study

ABSTRACT

Forty goats, aged from 2 to 5 months were subjected to two different immunization protocols with a vaccine containing *Haemonchus contortus* gut membrane proteins H11/H-gal-GP to evaluate protection against *H. contortus* on pre-contaminated pastures. Goats were allocated to four groups of ten, three of them received their first vaccination before turnout. One group (V4) was then vaccinated at 4-week-intervals whereas another two groups (V6 and V6SEP) were vaccinated at 6-week-intervals. A control group (CTRL) remained unvaccinated. In May, after the second vaccination, all goats were turned out on pastures which had been previously contaminated with *H. contortus* eggs by seeder sheep for a period of six weeks. Goats of groups V4, V6 and CTRL were grazed together, whereas V6SEP was kept separately at an identical stocking rate. Clinical (PCV, FAMACHA, body weight), parasitological (faecal egg count, FEC) and serological (antibody titres) parameters were measured fortnightly. All goats were stabled in October, drenched with levamisole and two weeks later infected with 5000 L3 of *H. contortus* and slaughtered four weeks later for determination of abomasal worm burdens. Group mean FEC peaked 42–56 days after turnout. Significantly lower FEC were observed in V6SEP vs CTRL between D 28 and 70 ($p < 0.01$). Mean egg output of all groups decreased substantially and fluctuated at low levels until the end of the grazing period (D 154). Goats responded to vaccination with increasing antibody titres peaking after every booster. Mean worm burdens deriving from experimental infections were reduced by 89, 65 and 47% in groups V4, V6 and V6SEP, respectively, compared with the controls. The difference was significant for V4 ($p < 0.01$). Antibody titres measured 14 days before slaughter did not correlate statistically with the worm burdens. It was concluded that the vaccination protocol did not result in sufficient protection on pasture, as antibody titres were still low at the time the goats were exposed to larval contamination on pasture after turnout.



## 1. Introduction

Amongst gastrointestinal nematodes (GIN), *Haemonchus contortus* is one of the most economically important parasites in goats and sheep worldwide (Zajac, 2006). The clinical and economic losses are mainly associated with the consequences of blood-sucking activity of the preadult and adult stages. Current control strategies against GIN usually rely on repeated anthelmintic treatments. As a consequence, anthelmintic resistance in ovine and caprine GIN is widespread (Saddiqi et al., 2011; Papadopoulos et al., 2012). Various alternative strategies for GIN-control have been investigated, including nematophagous fungi, bioactive forage crops and breeding for genetic resistance (reviewed by Waller (2006)). Specific immunization is currently the most advanced approach for non-chemical control of *H. contortus*. Most studies have been carried out in sheep based on gut membrane proteins of adult *H. contortus* (Kabagambe et al., 2000; LeJambre et al., 2008), or recombinant vaccines (Cachat et al., 2010). Very few trials focused on vaccination of goats, all of them with somatic (Ruiz et al., 2004; Molina et al., 2012) or recombinant antigens (Han et al., 2012). The use of native hidden antigens based on the H11 and H-gal-GP complex has so far resulted in the highest protection in sheep (reviewed by Smith and Zarlenga (2006)). At present no published report is available on the use of native H11 and H-gal-GP complex in goats. Therefore a grazing experiment was performed with young goats to investigate different immunization regimes based on the H11/H-gal-GP antigen complex under the conditions of the Swiss midland region.

* Corresponding author.
*E-mail address:* hubertus.hertzberg@access.uzh.ch (H. Hertzberg).

http://dx.doi.org/10.1016/j.vetpar.2016.08.024
0304-4017/

## 2. Materials and methods

### 2.1. Goats

The experiment was performed with 40 castrated male Chamoix goats purchased from a local farm. The goats had been reared indoors and were fed hay and commercial pelleted rations after being weaned at the age of 8–10 weeks. They had free access to fresh water and mineralized salt. After arrival at the study site at April 2 (D-36) they were two to five months old, with an average of 106 days. Prior to the first samplings, the goats were adapted for one week to the new environment. During 4 weeks of adaptation to pasture-feeding (D-28 to 0), the goats were fed hay, weaned from pelleted food, and diet was continuously changed from hay to grass. One week after arrival, the majority of goats developed pneumonia and were treated twice within 14 days with 10 mg/kg body weight (BW) tilmicosin (Micotil® 300, Provet AG Switzerland) and 0.5 mg/kg BW meloxicam (Metacam® 20 mg, Boehringer Ingelheim GmbH Switzerland). After having been base-immunized in the breeding stable, at D −28 all goats were boostered against *Clostridium perfringens* infection (Ovilis® Heptavac P, Veterinaria AG Switzerland), followed by a further immunization at the end of August.

The animal experiments were carried out in accordance with protocols approved by the Cantonal Veterinary Office (No. ZG 57/12).

### 2.2. Pastures

The trial was conducted at the experimental farm of the Federal Institute of Technology of Zurich in the canton of Zug, Switzerland (47° 10′ 34.99″ N, 8° 25′ 35.02″ E; 444 m.a.s.l.) from April to November 2012. The experimental pastures had not been grazed by farm animals during the previous year. Before the goats were transferred, the experimental pastures had been contaminated for six weeks by four adult seeder sheep, each of which had been infected with 3000 third stage larvae ($L_3$) of a local *H. contortus* isolate. These sheep exhibited FEC between 2440 and 5040 during the seeding period.

### 2.3. Experimental design

At arrival the goats were ranked according to body weight, blocked and randomly assigned to four groups (V4, V6, CTRL, V6SEP) of 10 animals each (group average 19.9 kg). At the same occasion the goats were treated anthelmintically to eliminate potential infections with GIN. Anthelmintic treatment consisted of a combination of levamisole hydrochloride and triclabendazole (Endex® 8.75%, Novartis AG Switzerland), administered orally at a single dosage of 12 and 16 mg/kg BW, respectively. Levamisole was chosen as it was known to be fully effective against the selected *H. contortus*-isolate. In Switzerland, levamisole is only marketed as a combination with triclabendazole.

Absence of strongylid eggs in faeces was investigated by faecal egg counts and coprocultures. Goats were vaccinated with 5 μg of *H. contortus* gut membrane proteins in 1 ml QuilA adjuvant subcutaneously, a vaccine recently commercialized as Barbervax® (barbervax.com.au) in Australia. Prior to use, the vaccine was stored at 4 °C. Goats of groups V4, V6, V6SEP received a basic immunization before turnout at D-28 and 0. After turnout boosters were applied either every 4 (V4) or 6 weeks (V6 and V6SEP) for a total of 8 (V4) and 6 (V6, V6SEP) immunizations, respectively. Goats of CTRL-group remained unvaccinated. A packed cell volume (PCV) with values of less than 20% or a faecal egg count (FEC) of higher than 1000 eggs per gram (epg) were chosen as a threshold for administering a salvage treatment.

At May 8 (D 0) the animals were turned out on two adjacent pastures, designed for an average stocking rate of 125 square meters per goat. Groups V4, V6 and CTRL were grazed together on one pasture (P1), group V6SEP grazed separately (P2). The size of the paddocks was increased stepwise over the season, according to the availability of grass. The goats stayed on pasture permanently until October 9 (D 154) and were only brought into the stable for samplings and during one period of 4 days at the end of August, when the temperature during daytime exceeded 30 °C and goats were kept indoors during the day. Goats were only fed grass, except during the last 6 weeks at the end of the trial when they were fed hay indoors. In August, restricted to a period of two and a half weeks after they had started to lose weight, all goats were supplemented daily with 1 kg hay and 400 g commercial pelleted rations (UFA 763, UFA AG, Switzerland). For the last phase of the trial, at D 154 all animals were treated with levamisole/triclabendazole at a dose rate as described above and housed until slaughter (on D 196/197). Herewith the different vaccination protocols could be re-evaluated after the goats had experienced the same conditions of housing/infection-pressure. So at D 168, the animals of the three vaccinated groups received a final vaccination dose, and all goats were challenged orally with 5000 third-stage-larvae of the same *H. contortus*-isolate used for seeding the experimental pastures.

### 2.4. Sampling

All goats were sampled fortnightly from D -28 until D 168, then sampling was undertaken weekly until slaughter. At the sampling days, the general condition of each goat was clinically examined by a veterinarian, which included weighing with an electronic scale (accuracy 0.1 kg), and scoring for the degree of anemia (1–5) by examining the eye mucosa using the FAMACHA card (van Wyk and Bath, 2002). Individual faecal samples were collected directly from the rectum, and blood samples were collected from the jugular vein into sterile blank 9 ml Vacuette tubes and 2 ml Vacuette tubes containing EDTA, respectively. Despite all goats being boostered against *C. perfringens* infection, one goat died 3 days after the injection (D -25) because of peracute infection with *C. perfringens* Type D. Another goat died by asphyxiation 3 days after turnout as a consequence of a fencing accident during night. Data from those 2 goats were discarded from the calculations.

### 2.5. Parasitological techniques

FEC were performed using a modified McMaster method with a sensitivity of 50 epg (Schmidt, 1971). Eggs other than strongyles were categorized separately. For identification of infective larvae, coprocultures were made according to Eckert (1960). Pooled faecal samples of each treatment group were mixed with sawdust and incubated for 7 days at 27 °C. From each culture, 100 third-stage-larvae (L3) were morphologically differentiated according to the guidelines of MAFF (Ministry of Agriculture, 1986). Pasture contamination with infective larvae was determined according to Sievers Prekehr (1973), modified by Hertzberg et al. (1996). Every 6–8 weeks, the presence of lungworm larvae and liver fluke eggs was tested with the Baermann and sedimentation technique, respectively. After slaughter and exsanguination, the abomasa were ligated and removed immediately. The contents were washed thoroughly and filtered through a 200 μm sieve. A 10% aliquot was separated and fixed with formaline at a final concentration of 4%. Male and female adults and juveniles of *H. contortus* were counted under a dissecting microscope at a magnification of 50 and the total number calculated per individuum.