DNA vaccine encoding *Haemonchus contortus*
Actin induces partial protection in Goats

Ruofeng Yan, Jingjing Wang, Lixin Xu, Xiaokai Song and Xiangrui Li*

College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China

Abstract

Actin is a globular multi-functional protein that forms microfilaments, and participates in many important cellular processes. Previous study found that *Haemonchus contortus* actin could be recognized by the serum of goats infected with the homology parasite. This indicated that *H. contortus* actin could be a potential candidate for vaccine. In this study, DNA vaccine encoding *H. contortus* actin was tested for protection against experimental *H. contortus* infections in goats. Fifteen goats were allocated into three trial groups. The animals of Actin group were vaccinated with the DNA vaccine on day 0 and 14, and challenged with 5000 infective *H. contortus* third stage larval (L3) on day 28. An unvaccinated positive control group was challenged with L3 at the same time. An unvaccinated negative control group was not challenged with L3. The results showed that DNA vaccine were transcribed at local injection sites and expressed in vivo post immunizations respectively. For goats in Actin vaccinated group, higher levels of serum IgG, serum IgA and mucosal IgA were produced, the percentages of CD4+ T lymphocytes, CD8+ T lymphocytes and B lymphocytes and the concentrations of TGF-β were increased significantly (P<0.05). Following L3 challenge, the mean eggs per gram feces (EPG) and worm burdens of Actin group were reduced by 34.4% and 33.1%, respectively. This study suggest that recombinant *H. contortus* Actin DNA vaccine induced partial immune response and has protective potential against goat haemonchosis.

Keywords

*Haemonchus contortus*; Actin; DNA vaccine; Goats

Introduction

*Haemonchus contortus* is blood ingesting nematode of ruminants, causing major losses to the agricultural industry worldwide (Knox et al. 1993; Newlands et al. 2001). The widespread emergence of *H. contortus* resistant strains to anthelmintic drugs currently available (Wolstenholme et al. 2004) has dramatically accelerated the need for alternative, sustainable control measures. Among them, the immunization of small ruminants against *H. contortus* has been tested over the last decades utilizing different immunizing preparations (Knox and Smith 2001). Native antigens including cysteine proteases expressed at the microvillar surface of the parasite intestinal cells (Skuce et al. 1999), passage of adult *H. contortus* membrane bound extracts over a thiol sepharose affinity column resulted in a 24-fold enrichment of cysteine protease activity (TSPB) (Redmond and Knox 2004), integral membrane protein from the intestinal microvilli (H11) of the parasitic stages of *H. contortus* (Smith and Munn 1990; Munn et al. 1993a,b) were been proved for partial protective efficiency with reduced faecal egg output and worm burdens. However, for the difficulty to get enough native protein and the poor protection of recombinant proteins, no commercial vaccine against *H. contortus* was made up to date (Newton et al. 1999).

During the development of vaccines, DNA vaccines with effective potential candidates have particularly attracted close attention and represented a new approach for the control of infectious nematode. The direct injection of a naked plasmid DNA vaccine encoding a foreign antigen results in plasmid uptake and protein expression, leading to the induction of antigen-specific cell-mediated immune responses (CMI) and antibody-mediated immune responses (AMI) (Tang et al. 1992). The ability of DNA vaccines elicited immune responses had been proved to be effectively against some parasitic infections (Alarcon et al. 1999; Masih et al. 2011; Chen et al. 2012). DNA vaccine encoding *H. contortus* cysteine protease (HC58), glutathione peroxidase (Hc29), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aminopeptidase (H11) (Muleke et al. 2007a; Sun et al. 2011; Han et al. 2012;

*Corresponding author: lixiangrui@njau.edu.cn*
Zhao et al. 2012) were investigated for the protection efficiency, and 28–38% reduction in worm burden were achieved. In another relative research, the mean eggs per gram feces (EPG) and worm burdens were reduced by 45.7% and 51.1% after goats were vaccinated with DNA vaccine expression disorganized muscle family member (Dim-1) (Yan et al. 2013). These results indicated that the enzymes involving in metabolizing and the components of the worm’s cytoskeleton could be the target for vaccine candidates.

Actin is a globular multi-functional protein that forms microfilaments. It is found in all eukaryotic cells (exception of nematode sperm). Actin is the monomeric subunit of microfilaments and thin filaments, which were major components of the cytoskeleton for cells and contractile apparatus of muscle cells, respectively. It can be present as either a free monomer called G-actin or as part of a linear polymer microfilament called F-actin. Both of them are essential for such important cellular functions as the mobility and contraction of cells during cell division. Thus, actin participates in many important cellular processes, including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signalling, and the establishment and maintenance of cell junctions and cell shape (Robinson and Shoichiro 2006).

In previous research, a homologue of actin in *Haemonchus contortus* was detected by serum from infected goats when whole proteins of male and female adult *H. contortus* were analyzed by immunoproteomics and mass spectrometry techniques, implying that actin was an immunogenic protein in this nematode (Yan et al. 2010). After which, the full sequence of actin cDNA of *H. contortus* was cloned and the DNA vaccine was constructed (Yan et al. 2012). However, the immunoprotection of this vaccine was not known yet. In this study, the protective efficacy of this DNA vaccine against *H. contortus* was investigated in goats.

**Materials and Methods**

**Parasites**

The *H. contortus* strain used was originally obtained from Nanjing, Jiangsu Province, China, and maintained by serial passage in the laboratory. Feces were collected and cultured at 28°C to yield L3 for challenge infection. The resulted L3 was less than 1 month old for use and were stored in water at concentration of 1 000 ml⁻¹ at 4°C. Adult *H. contortus* worms were collected from goat abomasum as previously described (Muleke et al. 2007a,b; Sun et al. 2007).

**DNA vaccine and Vaccination protocol**

The DNA vaccine encoding *H. contortus* actin was constructed as previous description (Yan et al. 2012). Briefly, The ORF of actin gene was generated by RT-PCR and cloned into pMD-18T plasmid (Takara Biotechnology). The pMD-18T/Actin ORF and the pVAX1 vector (Invitrogen, Life Technologies) were treated with *EcoR* I and *Hind* III enzyme and Actin ORF was directionally cloned into the pVAX1 vector. Recombinant pVAX1/Actin vector was digested with identical restriction enzymes and sequenced by Invitrogen biotech (Shanghai, P. R. China) for identification. The constructed plasmids were transformed into *Escherichia coli* strain DH5α and cultured for 8 h at 37°C. The plasmids pVAX1/Actin acting as DNA vaccines were prepared using Qiagen Plasmid DNA Mid Kit (Qiagen, USA) according to the manufacturer’s instructions. The eluted products were dissolved in PBS (pH 7.4) at a concentration of 1 mg/ml and stored at −20°C until required.

Fifteen local bred goats (8–10 months) were raised indoors under nematode-free conditions. The paddock was cleaned every day and disinfected every three days during the experiment. The animals were allocated into 3 groups of 5 goats each, and balanced as much as possible for body weight (16 ± 4 kg) and sex (8 males and 7 females).

According to the permission for inoculating the goats with the DNA vaccine issued by the Animal Care and Ethics Committee of Nanjing Agricultural University (Approval No. 201009022), Actin group (*n* = 5, 2 males and 3 females) received i.m. 100 μg of Actin DNA vaccine (0.1 μg/μl) in 1ml of PBS pH 7.4. The 1 ml injection volume was equally divided between two injection sites in the thigh and shoulder muscles. A booster dose was repeated with the DNA vaccine after a 2-week period. Negative control group (*n* = 5, 3 males and 2 females) was unvaccinated and unchallenged with L3, but mock-vaccinated with 1ml of PBS pH 7.4. Positive control group (*n* = 5, 3 males and 2 females) was unvaccinated, but challenged with L3 infection and mock-vaccinated with 1ml of PBS pH 7.4. Actin group received two vaccinations at 2-week intervals, on the day 0 and day14. On the day 28, animals of Actin group and positive control group were orally challenged with 5000 infective *H. contortus* L3 larvae. All goats were slaughtered humanely on day 63 following protocols approved by the Animal Care and Ethics Committee of Nanjing Agricultural University (Approval No. 200709005).

**Detection the transcription and translation of DNA vaccine in muscle cell**

The transcription of Actin DNA vaccine at local muscle in injection sites was determined 7 days after the first vaccination by RT-PCR utilizing gene specific primers with the methods as described previously (Muleke et al. 2007a). In brief, 0.5 g muscle tissue each was scrupulously taken from injection sites of Actin and negative control group by surgery after anaesthesia with 2 mg/kg xylidnothiazoline. The RNA pellet was suspended in 20 μl diethylpyrocarbonate (DEPC; Invitrogen)-treated water, then treated with DNase (Invitrogen). The composition of PCR reaction solutions was 16.5 μl water, 2.5 μl 10× PCR buffer, 2.5 μl deoxynucleotide triphosphate (dNTP) mixture (2.5 mM each of 4 dNTPs, initial concentration), 2 μl...
primers (20 pM each), 1 µl cDNA and 0.5 µl Taq DNA polymerase (5 U/µl). PCR conditions were 1 cycle of 94°C for 3 min, 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, then 1 cycle of 72°C for 10 min.

Ten days after boosting, a surgical biopsy of muscle (approximately 0.5 g) was collected from each injection site of goats in Actin and negative control groups with the methods as described previously (Muleke et al. 2007a). In brief, the goats were locally anesthetized by xylidnothiazoline at a dose of 2mg/kg. After 15 min, the skin and fascia covering the anterior compartment was incised and reflected to expose the muscle. A small femur fiber bundles approximately 2 cm in length was isolated. Antibiotic was given to avoid bacterial contamination after suturation. Then, the samples were grinded and treated with RIPA solution (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% Nonnized P-40, 0.1% SDS) for 3 h. After centrifugation at 10000 g for 5 min, the supernatant was collected and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to nitrocellulose membranes (Amersham). The membranes were probed with rat anti-recombinant actin serum as the first antibody (produced as Yan et al. 2012 described), followed by incubation with peroxidase-conjugated goat anti-rat antibody (IgG-HRP) (Booster Technology) and a colorimetric reaction with 3,3’,5,5’-Tetramethylbenzidine tetrahydrochloride (DAB) kit (Boster Bio-technology).

Flow cytometry

Lymphocyte subsets in peripheral blood mononuclear cells were determined utilizing a flow cytometry assay following Sun’s methods (Sun et al. 2007). For preparation of blood mononuclear cells, blood was collected from the jugular vein in heparin (0.2 mg/ml of blood) on day 0, 14, 28, 45 and 63 of the experiment. Erythrocyte lysis was done on pelleted blood cells with pre-warmed ammonium chloride (pH7.4) at 37°C, followed by three washes in PBS (pH7.4). Isolated leucocytes were resuspended to a concentration of 1×10⁶ cells in 100 µl volume of PBS (pH7.4). The cells were centrifuged at 1200 g for 5 min and the medium was removed. PE-conjugated monoclonal antibodies to CD4 (GC50A1), CD8 (BAQ111A), CD2 (MUC2A) and B lymphocytes (B-B2) (BAQ44A) (Caltag Laboratories) were added, respectively. The cells and the antibodies were mixed in 1 ml PBS (pH7.4) and placed in the dark for 15 min. Unbound antibodies were removed by washing with 2% FCS in PBS (pH7.4). The fluorescein labeled secondary antibody (goat anti-mouse IgG1, FITC conjugate antibody) (Caltag Laboratories) was diluted 1/40 in PBS (pH7.4). 10 µl of the diluted secondary antibody was added to the cells. The cells and antibody were mixed and incubated dark for 15 min. The cells were then washed once in 2% FCS in PBS and again in FACS Buffer (5% FCS and 1% sodium azide in PBS). After the final wash, the cells were re-suspended in 500 µl of FACS buffer and 500 µl of 1% paraformaldehyde in PBS. Stained cells were analyzed using a FACScan flow cytometer equipped with CellQuest software (Becton Dickinson, Mountain View, CA).

Preparations of serum, mucosal surface (MS) and mucosal homogenate (MH) samples

Goats were bled from the jugular vein at 0, 14, 24, 28, 33, 43, 53 and 63 days of the experiment. At each bleeding, 5ml of blood was collected from each goat into sterile plain universal bottles and kept at 4°C overnight to clot. The supernatant was centrifuged at 3000 g for 20 min. Separated serum was stored at −20°C until used.

The preparations of MS and MH were carried out following Muleke’s method (Muleke et al. 2007a). On the day 63, mucus was collected by gently scraping the mucosal surface of an abomasum strip (about 2 cm wide and 20 cm long) with glass microscope slides. Each gram of scraped material was incubated overnight at 4°C in 3 ml of 0.1 M PBS pH 7.4 containing 0.005 M EDTA, 0.005 M PMSF and 0.01% soya bean trypsin inhibitor (Sigma, USA). Then the mixture was centrifuged at 10000 g for 30 min and the supernatant, designated as mucosal surface (MS) was stored at −20°C until used. The MS samples were used to detect the IgA secreted to the mucosa surface.

Mucus and surface epithelial layer was collected by vigorously scraping mucosal surface of the abomasums with glass microscope slides. The scraping was homogenized in three volumes of cold 0.1 M PBS (pH 7.4) containing 0.005 M EDTA, 0.005 M PMSF and 0.01% soya bean trypsin inhibitor. The homogenates were centrifuged at 10000 g for 30 min and the supernatant, designated as mucosal homogenate (MH) was stored at −20°C until used. The MH samples were used to inspect the IgA secreted in the mucosa tissues.

ELISAs for detection of antibodies in serum, MS and MH

The titers of serum IgG were determined by indirect ELISA with the methods as described previously (Sun et al. 2007). Microtitre plates (Bethyl Laboratories, Inc., USA) were coated overnight at 4°C with 1µg/ml recombinant actin protein of prokaryotic expression (described as Yan et al. 2012) in 0.06 M carbonate buffer (pH 9.6). After washed with PBS containing 0.05% (v/v) Tween-20, plates were blocked with 1% (w/v) skimmed milk in PBS for 1.5 h at room temperature. Serum samples diluted at 1:1000 in blocking buffer was added to each well, and incubated for 1 h at 37°C. Plates were incubated with horseradish peroxidase-conjugated rabbit anti-goat IgG (Southern Biotechnology Associates, Inc., USA). The 3, 3’, 5’, 5’-Tetramethylbenzidine (TMB)- Peroxidase substrate system (Beyotime institute of biotechnology, P. R. China) was used for the colorimetric reaction. Plates were read at an optical density of 450 nm in the Model 550 microplate ELISA reader (BIO-RAD, Japan). Standard positive and negative controls were included on each plate. The results were expressed as optical density of the absorbance. The concentra-
tions of serum IgA, MS-IgA and MH-IgA were determined utilizing indirect ELISA with the “Goat IgA ELISA Quantita-

tion Kit” (Bethyl Laboratories, USA) according to manufacturer instructions. The dilution for serum and conjugate rabbit anti-goat IgA-HRP was 1:1000 and 1:20,000, respectively. The dilution for MS and MH samples was 1:10 each.

Determination of serum cytokine concentration

On day 0, 14, 28, 45 and 63 of the experiment, the concentrations of IL-4, interferon-γ (IFN-γ), transforming growth factor-β (TGF-β) and IL-22 in serum were detected utilizing indirect ELISA with the “Goat cytokine ELISA Quantitation Kits” (Catalogue numbers: I042-01 for IL-4, I015-51 for IFN-γ, I015-52 for TGF-β, I081-01 for IL-22. GBD Laboratories, USA) according to manufacturer instructions.

Eggs per gram feces (EPG) and abomasum worm counts

Fresh fecal samples were collected rectally from each goat and, thereafter, at 50, 52, 54, 56, 58, 60 and 62 days of the experiment. The EPG was determined by the modified McMaster method, and the reduction of EPG was calculated as follows.

\[
\text{Positive – Actin} \times 100\%
\]

The number of worms in the abomasum of each goat was determined on day 63. The abomasal contents were collected and the mucosa were scraped and washed with warm 0.9% sodium chloride to detach any adhering worms. All parasites from the abomasal contents and the mucosal surface were picked out, collected, counted and sorted according to their sexes. The reductions in total worms were calculated using the same method in EPG.

Differential cell counts

Blood samples were collected by jugular venipuncture into evacuated glass tubes (Becton Dickinson, Oxford, UK) containing EDTA anti-coagulant for differential blood cell count on day 0, 14, 28, 45 and 63 of the experiment. Differential cell counts were carried out with an automated electronic cell counter (HYCEL DZANA Model 5, France).

Statistical analysis

Statistical analysis was carried out using SPSS statistical package (SPSS for Windows 11.5, SPSS Inc., Chicago, IL, USA). When data conformed to normal distributions, analysis was by ANOVA in General Linear Model on raw values and the results were summarized as arithmetic means with standard deviation. While data did not conform to normal distribution, appropriate logarithmic transformations were adopted prior to analysis and all residuals for ANOVA were checked for approximately normal distribution. The difference of EPG and abomasal worm counts between Actin group and the positive control group were also analyzed by t-test. Differences between groups were considered significant at P<0.05.

Results

Transcription and translation of DNA vaccines in muscle tissue

The results of RT-PCR indicated that Actin DNA vaccine was transcribed 7 days after the first vaccination in vaccinated group goats (Fig. 1a, lane 1). The target fragment of 1131 bp was lacking in the muscle sections of negative control group goats (Fig. 1a, lane 2).

Western blotting indicated that Actin was expressed 10 days after booster injection in vivo (Fig. 1b, lane 1). In contrast, muscle tissue sections obtained from negative control group goats, failed to react with rat anti-recombinant Actin serum (Fig. 1b, lane 2).

Lymphocyte subsets in peripheral blood mononuclear cells

After vaccination, the percentage of CD4+ T lymphocytes in Actin group increased and was significantly higher than that in both control groups at day 14 and 28 (P<0.05) (Fig. 2a). The percentage of CD4+ T lymphocytes in both control groups didn’t show any notable difference. Following L3 larvae challenge, the percentage of CD4+ T lymphocytes in positive con-
control goats increased dramatically and was significantly higher than that in the negative control group. The CD4+ T lymphocyte level remained substantially low throughout the experiment in the negative control group.

After boosting immunization, the percentage of CD8+ T lymphocytes increased significantly at day 28 (P<0.05) (Fig. 2b). No difference was found in both control groups before L3 larvae challenge. However, after L3 larvae challenge, CD8+ T lymphocytes increased significantly in positive control group at day 45 and 63 (P<0.05). The level of CD8+ T lymphocyte in the negative control group remained substantially low throughout the experiment.

The percentages of B lymphocytes in Actin group went up steadily from day 0 to day 63, and was significant higher than that in control groups at 28d (P<0.05). After L3 larvae challenge, B cells in the positive control group increased dramatically from day 45, reached the peak at day 63, and there was no statistical difference of B cell levels between Actin group and positive control group. The percentages of B lymphocytes in negative control group maintained at a stable level during the whole experimental stage (Fig. 2c).

**IgG and IgA levels in serum and abomasum**

Group mean (±S.D.) serum IgG levels for Actin group reached peak values at day 28 and remained significantly high until the end of study (P<0.05) (Fig. 3a). In contrast, serum IgG level for negative control group was substantially lower. The serum IgG level for positive control group rose after L3 larvae challenge.

From day 14 to day 63, serum IgA levels of Actin group differed significantly (P<0.05) when compared with those of the control groups (Fig. 3b). While the serum IgA kept stable at low levels in both control groups from day 0 to day 28. After L3 challenge, the IgA levels in positive control group increased and reached peak values at day 53.

Means of MS-IgA for Actin group were significantly higher than that for positive control group, while MH-IgA for

![Graphs showing lymphocyte counts](image-url)
DNA vaccine against H. contortus infection in goat

Actin group differed significantly from those of both control groups (P<0.05) (Fig. 3c).

Concentration of serum cytokines

Before L3 larvae challenge, no prominent change of IL-4 and IFN-γ concentration could be observed in all groups (Fig.4a and 4b). However, after L3 larvae challenge, there were marked rises of IL-4 concentration in both Actin and positive control group goats on day 63 compared with the negative control group (P<0.05). The concentration of IL-4 and IFN-γ in negative control group goats maintained at a constantly stable level during the whole experiment. On the contrary, IFN-γ concentration of Actin and positive control group goats declined slightly after L3 larvae challenge (Fig.4b).

No statistical difference of TGF-β concentration in all experimental groups could be observed at day 0 and 14 (Fig. 4c). At day 28, the concentration of TGF-β in Actin vaccinated group increased dramatically and significantly differed from that of the control groups. After artificial infection, TGF-β concentration in both challenged groups was higher than that of unchallenged negative control group on day 45 and 63 (P<0.05), but there was no notable difference of TGF-β concentration between Actin and positive control group.

No difference was observed for IL-22 concentrations in all groups at day 0 and 14 (Fig. 4d). After artificial infection, IL-22 concentrations in Actin and positive control groups were slightly decreased from day 45 to day 63 and significant lower than the unchallenged negative control group at day 63.

Differential cell counts and hemoglobin concentration

Before L3 challenge, the peripheral eosinophils, neutrophils and monocytes count of all groups didn’t show any difference (Fig. 5a, 5c and 5d). But after L3 larvae challenge, the peripheral eosinophil counts of Actin and positive control group increased and were significantly higher than that of the negative control group (P<0.05) which sustained a relatively stable
level during the experiment. The basophile in peripheral blood cell keep stably in the experiment and no significant difference among the groups (Fig. 5b).

There was a reduction (P<0.05) in the concentration of hemoglobin in Actin and positive control groups after L3 larvae challenge. No phanerous difference was observed between Actin and positive control group at day 45, but the difference was significant at day 63 (Fig. 5e). In contrast, there was no significant change in the hemoglobin density values of the worm free goats of negative control group.

**Table 1. Abomasal worm counts**

<table>
<thead>
<tr>
<th>Group</th>
<th>Female</th>
<th>Male</th>
<th>Total worms</th>
<th>Reduction of worm burdens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Positive control</td>
<td>432.8 ± 101.15</td>
<td>380.8 ± 75.16</td>
<td>813.6 ± 175.86</td>
<td>–</td>
</tr>
<tr>
<td>ACT group</td>
<td>285.2 ± 59.77*</td>
<td>259 ± 53.63*</td>
<td>544.2 ± 113.01*</td>
<td>33.1%</td>
</tr>
</tbody>
</table>

Goats vaccinated by pVAX1/Actin and challenged = ACT group; goats injected only with PBS and challenged = Positive control group; goats uninfected and unimmunized = Negative control group. The animals were vaccinated on days 0 and 14, challenged with 5000 *Haemonchus contortus* L3 on day 28, and slaughtered humanely on the day 63. Values bearing a different superscript signal. (*) within a line differ significantly from one another (P < 0.05).
Faecal worm egg counts and worm burdens

The EPG in the vaccinated versus unvaccinated goats after challenge was shown in Fig. 6. The results of faecal egg counts indicated that, egg shedding in Actin and positive control group began at day 50 of the experiment, and increased gradually to the peak at day 60. In contrast, negative control group goats did not shed eggs during the whole experiment period. The average EPG for Actin group was reduced by 34.4% (P<0.05) in comparison to that for positive control group.
Worm counts were listed in Table I, which showed notable reductions of worm burdens in Actin group. The reductions of female, male and total worms in Actin group were 34.1% (P<0.01), 32.0% (P<0.01), 33.1% (P<0.01) compared to that of positive control group.

Discussion

Vaccines against *H. contortus* have been pursued and advocated for decades. Several partially protective antigens have been identified, none of which has made it to product (Newton *et al.* 1999; Smith *et al.* 1990). In the present study, goats vaccinated with pVAX1/Actin were partially protected with reductions in egg output and abomasal worm burden of 34.4% and 33.1%, respectively. This could be a successful example of a DNA-based strategy that has provided a modest response. However, this is the preliminary study. Repeat trials should be done in the future to get more confidence of the results.

A difference of susceptibility to gastrointestinal nematodes between male and female, in sheep and goats was well documented (Herd *et al.* 1992; Barger *et al.* 1993). In this experiment, there are male and female in each group, further study in only male or female goats should be done in the future. However, the results in the current study could better represent the real efficacy in the practice of using this vaccine, for the natural infections of the parasites often occurred in both genders of animals. Five animals in each group may not sufficient for the vaccination. And more animals or replicated experiment should be done in the further investments. However, the similar goat numbers were often used in assessing the vaccines against the virus and parasites (Domínguez-Torano *et al.* 2003; Muleke *et al.* 2007a; Sun *et al.* 2011; Zhao *et al.* 2012; Molina *et al.* 2012).

In DNA vaccine, the vector may play some roles in the protection. In our experiment, pVAX1 was applied. This vector was consistent with the Food and Drug Administration (FDA) document (`Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications’, published December 22, 1996, Docket no. 96N-0400). Features of this vector allow high-copy number replication in *E. coli* and high-level transient expression of the protein of interest in most mammalian cells. So far, it was announced to be the safest vector in DNA vaccine by FDA, for the minimal possibility of chromosomal integration and less likely to elicit allergic responses. Theoretically, the immune responses by the vector pVAX1 could be ignored. In the previous study, it was reported that experimental animals injected with pVAX1 plasmids did not show any change in protective efficacy against infectious parasites compared with challenged positive controls, indicating that effects by DNA vaccines were not attributed to non-specific factors related to plasmids (Song *et al.* 2009, 2010). No difference between pVAX1 control and blank control in mice was found in recent studies (Chen *et al.* 2012; Zhou *et al.* 2012).

Previous study showed that high specific IgG titre was related to the host defense against *H. contortus* challenge. It was found that cathepsin B cysteine proteases enriched thiols-Sepharose affinity chromatography fraction created rich IgG titres and induced an intense reduction in worm burdens and egg output of 47% and 77% respectively (Knox and Smith 2001). Some other evidences also supported this view (Munn *et al.* 1993a,b; Smith *et al.* 1994; Sun *et al.* 2007). In this study, animals in Actin immunized group presented significantly higher IgG titres, notably following booster vaccination as compared to unvaccinated control group goats. These findings were in agreement with Fengbin’s report, in which, actin protein in *H. contortus* was found to be easily recognized by anti-serum of goats infected with *H. contortus* (Yan *et al.* 2010). The results were consistent with previous reports.

Fig. 6. Dynamics of egg shedding. EPG was expressed as mean ± SD. Goats vaccinated by pVAX1/Actin and challenged = ACT group; goats injected only with PBS and challenged = Positive control group; goats uninfected and unimmunized = Negative control group. Samples were collected and counted at two days interval since 50 days of the experiment (22 days after L3 larvae challenge).
A close association between abomasum IgA and worm burdens was observed (Amarante et al. 2005). Similar results were reportedly associated with reduced percentage L3 for O. circumcincta in sheep (Stear et al. 1995), and O. ostertagi in calves (Geldhof et al. 2002). These researches suggested that IgA might play an important role in the protection against H. contortus and other gastrointestinal nematodes by parasite expulsion. The same phenomenon was observed in this study.

Studies in sheep showed that CD4+ and CD8+ T cells played a critical role in regulating the immune response to H. contortus (Gill et al. 1993; Karanu et al. 1997), and similar results were also obtained in goats (Sun et al. 2007). Our results were consistent with the reported results. Eosinophils may play an important role in immune responses against helminth infections and are often associated with the expression of resistance to the parasites in sheep (Pfeffer et al. 1996; Balic et al. 2000). In Huntley’s study, it was found that no differences were detected between the numbers of circulating or tissue eosinophils in sheep and goat (Huntley et al. 1995). In this research, higher numbers of eosinophils in the peripheral blood of vaccinated Actin group were found when compared with the positive control group. These findings concurred with the general observations of close association between eosinophils and resistance to helminth parasites (Yacob et al. 2004). Eosinophil recruitment is hypothesized to be dependent on cytokines secreted by CD4+ Th2 cells (Winter et al. 1997; Meeuwen et al. 2000). Thus, the variation trends of percentage of CD4+ T lymphocytes and the concentration of eosinophils in the present study fitted well within this hypothesis. Antigens produced by helminths are thought to be strong inducers of reagins and hemo-cytotropic IgG elements which, together with eosinophils, are responsible for antibody-dependent and cell-mediated cytotoxicity (ADCC) (Butterworth et al. 1985). The unvaccinated worm free goats of negative control group had fewer eosinophil levels, suggesting that the eosin staining cells indeed play roles or have their numbers stimulated as a byproduct of the immune process.

In this study, it was found that vaccinated with DNA encoding Actin gene could induce both humoral and cellular immune response. However, no difference was found in CD4+ T lymphocytes, B lymphocytes and eosinophils between Actin group and positive control group after L3 challenging. This may indicate that L3 challenging could induce dominant immune response rather than that by Actin DNA vaccine. Another possibly reason could be that more antigens were excreted/secreted after L3 challenging, and related immune responses were stimulated. In previous study, the same phenomena were observed (Sun et al. 2011; Zhao et al. 2012). However, the accurate mechanisms should be studied in the future.

Acknowledgments. This work was supported by the Fundamental Research Funds for the Central Universities (KYZZ01315) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References


**Received:** June 28, 2013  
**Revised:** May 16, 2014  
**Accepted for publication:** July 31, 2014