Larvicidal activity of *Bacillus thuringiensis* var. *israelensis* Cry11Aa toxin against *Haemonchus contortus*

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SUMMARY

Effective control of gastrointestinal parasites is necessary in sheep production. The development of anthelmintics resistance is causing the available chemically based anthelmintics to become less effective. Biological control strategies present an alternative to this problem. In the current study, we tested the larvicidal effects of *Bacillus thuringiensis* var. *israelensis* Cry11Aa toxin against *Haemonchus contortus* larvae. Bacterial suspensions [2×10^8 colony-forming units (CFU) g^{-1} of the feces] of *B. thuringiensis* var. *israelensis* and recombinant *Escherichia coli* expressing Cry11Aa toxin were added to naturally *H. contortus* egg-contaminated feces. The larvae were quantified, and significant reductions of 62 and 81% ($P < 0{\cdot}001$) were, respectively observed, compared with the control group. A 30 mL bacterial suspension (1×10^8 CFU mL^{-1}) of *B. thuringiensis* var. *israelensis* and recombinant *E. coli* expressing Cry11Aa toxin were then orally administered to lambs naturally infected with *H. contortus*. Twelve hours after administration, feces were collected and submitted to coprocultures. Significant larvae reductions ($P < 0{\cdot}001$) of 79 and 90% were observed respectively compared with the control group. The results suggest that the Cry11Aa toxin of *B. thuringiensis* var. *israelensis* is a promising new class of biological anthelmintics for treating sheep against *H. contortus*.

Key words: biological control, Cry11Aa, *Bacillus thuringiensis* var. *israelensis*, *Haemonchus contortus*.

INTRODUCTION

Helminth infection is a major limiting factor for sheep production around the world. The financial impact of these infections can be observed in the costs of veterinary services, reduced animal performance and even death. *Haemonchus contortus* is the main parasite responsible for this burden in many parts of the world (O'Connor *et al.* 2006). Through the years, *H. contortus* has been controlled principally with chemicals anthelmintics; however, the incidence of anthelmintic resistance is increasing among the population (Gasser *et al.* 2008; Van den Brom *et al.* 2015). To date, this parasite has acquired resistance to practically every major drug class available on the market. Drug resistance is now threatening sheep production around the world (Van Wyk *et al.* 1999; Cezar *et al.* 2010; Medeiros *et al.* 2014).

We have previously shown that various *Bacillus* isolates are highly toxic *in vitro* and *in vivo* to *H. contortus* larvae, suggesting that this group of bacteria can be used as a promising biological anthelmintic (Sinott *et al.* 2012, 2014). *Bacillus thuringiensis* is a Gram-positive bacterium that produces crystal inclusions upon sporulation. These inclusions are comprised Cry and Cyt proteins, which are toxic to a wide range of insects species among the orders *Lepidoptera*, *Diptera* and *Coleoptera* (Bravo *et al.* 2007; Pardo-López *et al.* 2013; Soberón *et al.* 2013), as well as other invertebrates of the phylum Nematoda (Marroquin *et al.* 2000). Commercial formulations, which generally consist of a mixture of crystal proteins and bacterial spores, are widely used for insect control (Bravo *et al.* 2011).

The use of *B. thuringiensis* as an anthelmintic presents certain advantages over traditional chemotherapeutic approaches, including an absence of chemical residues associated with its use, high target specificity and low mammalian toxicity (Schnepf *et al.* 1998; Siegel, 2001). *Bacillus thuringiensis* var. *israelensis* is toxic to the larval stage of *H. contortus* (Sinott *et al.* 2012), and there is potential for its use as a biological anthelmintic that targets the free-living larval stages developing in pasture. Biological strategies have the potential to reduce the levels of larvae in the pasture, where higher parasite numbers are present. The current study aimed to examine the potential of *B. thuringiensis* var.

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israelensis Cry11Aa toxin to control the larval stage the nematode *Haemonchus* sp. in sheep livestock.

MATERIALS AND METHODS

Bacillus thuringiensis *var*. israelensis

Spores of the strain *B. thuringiensis* var. *israelensis* (from the collection of the Department of Microbiology and Parasitology, Federal University of Pelotas) were streaked onto a BHI (Brain Heart Infusion – Acumedia) agar plate and grown for 24 h at 28 °C. Single colonies from the plate were used to inoculate 50 mL of NYSM (Yousten, 1984) medium and were grown at 28 °C with rotary shaking at 150 rpm for 72 h. Starter cultures were used to inoculate a flask containing 500 mL of NYSM media and then cultured at 28 °C with rotary shaking at 150 rpm for 72 h to ensure complete sporulation.

Construction of the expression vector

Bacillus thuringiensis genomic DNA was extracted using the Illustra Bacteria Genomic Prep Mini Spin Kit (GE – Healthcare) following the manufacturer's protocol. The sequence that contained the gene *cry11Aa* was amplified by PCR using the set of primers Cry11Aa Forward 5′ – GGG GAT CCA TGG AAG ATA GTT CTT TAG – 3′ and Cry11Aa Reverse 5′ – CCG GTA CCC TAC TTT AGT AAC GG – 3′. The primers were constructed based on a sequence obtained from GenBank (access number: AL731825.1), using the program Vector NTI (Invitrogen). The Taq DNA polymerase (Invitrogen) was used in the PCR. The amplification reaction was performed in a final volume of 25 μL containing 0·3 μL of Taq DNA polymerase (5 U μL^{-1}), 2·0 μL 10× PCR buffer, 1·0 μL 50 mM MgCl2, 0·5 μL dNTPs, 1·0 μL, 10 pM μL^{-1} of the forward and reverse primers, 2 μL template (solution containing 300 ng DNA) and sterile water to complete the volume of the reaction. Amplification was performed under the following conditions: 95 °C for 5 min (1 cycle), 94 °C for 50 s, 59 °C for 50 s, 72 °C for 2 min (32 cycles) and 72 °C for 3 min (1 cycle). The reaction was performed in a thermocycler (Eppendorf Mastercycle). The PCR products were cleaved with the *Bam*HI and *Kpn*I restriction enzymes before ligation of the fragment into the pAE vector, which had been digested with the same enzymes. The pAE plasmid containing the DNA sequence of the *cry11Aa* gene, without the stop codon, was extracted and sequenced to confirm the sequence and its frame with polyhistidine-tag. The recombinant clones obtained were named pAE-*cry11Aa* and were used for expression studies. *Escherichia coli* TOP10 cells were grown on Luria–Bertani (LB)-agar containing ampicillin (100 μg mL^{-1}) for solid culture and were used for plasmid maintenance using standard transformation protocol heat shock (Sambrook *et al*. 1989).

Culture growth conditions and protein purification

The *B. thuringiensis* bacteria were grown to sporulation on NYSM agar plates at 28 °C, harvested into water, and centrifuged (10 000 × ***g*** for 10 min, 4 °C). The pellet was washed twice in water to remove cell debris and secretory products. It was then suspended in water, and the volume adjusted to an OD_{600} of 0·5 and these suspensions were stored at −70 °C.

Escherichia coli BL21 (DE3) C43 was transformed with pAE-*cry11Aa* and grown in LB medium containing ampicillin (100 μg mL^{-1}) at 37 °C for 16 h. An aliquot of the 5 mL culture was used to inoculate 500 mL of the same medium, and the *E. coli* BL21 were grown under the same conditions until an OD_{600} of 0·5–0·6 was reached. Expression of recombinant protein Cry11Aa was induced by the addition of 0·3 mM IPTG (Isopropyl β-D-1-thiogalactopyranoside) for 3 h at 37 °C. Cell aliquots were collected by centrifugation for analysis by SDS–PAGE, Western blot and larvicidal assays. Soluble recombinant proteins were purified by affinity chromatography, where each Akta Wash supernatant (approximately 15 mL) was applied through a 1 mL Ni–Sepharose Hi Trap chelating column (GE Healthcare) that was previously equilibrated with its respective solvation buffer, following the manufacturer's guidelines. The Ni^{+} column was washed with 5 mL of the respective Akta Wash, and the protein was eluted with 1 mL aliquots of Elution Buffer (NaH_2PO_4, 0·234% and NaCl, 2·92%) with differing concentrations of Imidazole (at 10, 20, 30, 40 and 100%). Fractions of the 1 mL elution were collected. All flow-through portions were saved and applied to 12% SDS–PAGE. The elution fractions containing the protein were confirmed by SDS–PAGE. The purified rCry11Aa was examined by SDS–PAGE and Western blotting and was quantified at A_{280} according to a standard curve that was established using bovine serum albumin (BSA) on 12% SDS–PAGE.

For the SDS–PAGE, cell pellets from 1 mL of the induced culture were added to 100 μL of 2× protein loader buffer (4% sodium dodecyl sulphate, 20% glycerol, 120 mM Tris–HCL, pH 6·8, 10% 2-mercaptoethanol and 0·3% Bromophenol Blue). These were then incubated for 10 min at 99 °C for protein denaturation. Aliquots of 20 μL of each sample were loaded onto 12% SDS–PAGE. The gels were stained with Coomassie Brilliant Blue (CBB) or used to transfer the proteins to nitrocellulose membranes to perform the western blot. The nitrocellulose membranes were blocked with 5% skim milk powder in phosphate-buffered saline (PBS) at 37 °C agitating for 1 h, and then washed

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twice with PBS-T (PBS containing 0·1% Tween 20; pH 7·4) for 5 min. Membranes were incubated with the primary antibody—mouse monoclonal anti-His (C-term) (Invitrogen), 1:6000—for 1 h at 37 °C. The membrane was washed twice with PBS-T and incubated with peroxidase-conjugated rabbit anti-mouse IgG (1:6000) in PBS-T for 1 h at 37 °C. The membranes were then washed three times with PBS-T. The protein bands were visualized using a developing solution (5 mL of Milli-Q water; 5 mg of DAB – 3,3′-diaminobenzidinetetrahydrochloride; 0·75 mg of NH_4). Incubation in this solution proceeded until well-defined bands appeared on the nitrocellulose membrane (5–10 min), which was then washed in sterile Milli-Q water and photo-documented.

To estimate the protein concentration in the bacterial suspension, 20 μL [10^8 colony-forming units (CFU) mL^{-1}, *B. thuringiensis* and *E. coli*] was separated by SDS–PAGE (12%) and then visualized by CBB staining. Protein concentrations were than estimated by using a protein assay kit (Bio-Rad Laboratories) with BSA as the standard, using the image analysis software *ImageJ* (http://imagej.nih.gov/ij/).

Parasites

Feces from naturally gastrointestinal nematodes-infected male lambs (*Corriedale*, approximately 5 months old) were collected directly from the rectum. The number of nematodes eggs per gram (EPG) was determined using the Gordon and Whitlock (1939) technique, and only those samples containing more than 1000 EPG were used. It was estimated that 70–80% of the incubated eggs would hatch under favourable conditions, and this was confirmed during the experiments. To obtain the larvae, coproculture was performed using the Roberts and O'Sullivan (1950) technique using 4 g of fecal samples, held for 7 days in an incubator at 28 °C and relative humidity above 80%. The larvae (L3) were recovered, and then identification and counting performed. Collection of nematodes eggs from the donor sheep was conducted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles *et al.* 1992). The *Haemonchus* sp. larvae were the most prevalent in the coproculture, accounting for approximately 80% of the genus present, identified using the guidelines from Ueno and Gonçalves (1998).

Larvicidal effect of Cry11Aa

Coproculture adapted from the Roberts and O'Sullivan (1950) method was used. Briefly, 4 g of feces containing ⩾1000 EPG were inoculated with 2 mL of a bacterial suspension containing ~1×10^8 CFU mL^{-1} and treatments were as follows: (1) *B. thuringiensis*, (2) transformed *E. coli* expressing rCry11Aa, (3) non-transformed *E. coli* and (4) water as the control. Four containers for each treatment were used, and the experiment was repeated three times. After 7 days of incubation at 28 °C and relative humidity at 80%, larvae (L3) were recovered using the Roberts and O'Sullivan (1950) technique, and counted under a microscope at 40 × magnification (Olympus, Cx21 model). To estimate the efficiency in larvae reduction we used the formula $R = 100\ (1-T/C)$, where R is the reduction (larvicidal effect), C is the number of larvae in the control, and T is the number of larvae in the bacteria treated group (Coles *et al.* 1992).

Administration of Cry11Aa to sheep naturally infected with Haemonchus contortus

Sixteen male *Corriedale* sheep (~5 months old, maintained on native grazing, with a stool score of ⩾1000 EPG, positive for *H. contortus*) were divided in four groups with four animals each. All treatments were administered orally, and the groups were distributed as follows: A 30 mL bacterial suspension containing ~1×10^8 CFU mL^{-1} of: (1) *B. thuringiensis*, (2) transformed *E. coli* expressing rCry11Aa, (3) non-transformed *E. coli* and (4) water as the control. The fecal samples (8–10 g) were collected directly from the rectum at 12 h post-administration and larvicidal effects were evaluated by coprocultures as described above. Ethical approval for the use of sheep to provide feces for *in vitro* experiments was obtained from the Federal University of Pelotas, Animal Ethics Committee number CEEA 9118.

Statistical analysis

The percentage of total larval count reductions was determined using the method described by Coles *et al.* (1992), which uses the following formula: percentage reduction = $100 \times (1-T/C)$, where T and C are the geometric means in the treatment and control groups, respectively, on day 7 post-treatment. The total larval counts were analysed by analysis of variance (ANOVA) and by the Tukey's test, and P-values of $<0{\cdot}05$ were considered significant.

RESULTS

Expression of recombinant protein

The expression of Cry11Aa from *B. thuringiensis* var. *israelensis* by *E. coli* BL21 (DE3) C43 was confirmed by SDS–PAGE and Western blotting analysis using anti-6 × His Tag Mab. We observed a band at the expected size of ~72 kDa, which was similar to that of the *B. thuringiensis* var. *israelensis* spore/crystal suspension visualized by SDS–PAGE (Fig. 1).

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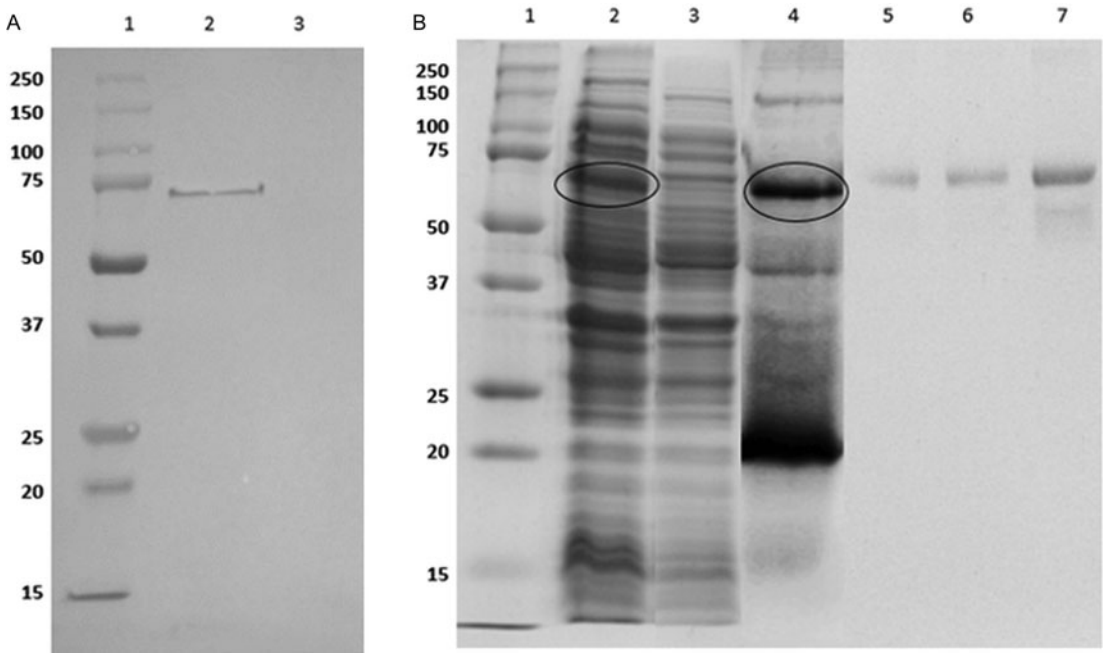

Fig. 1. Recombinant Cry11Aa characterization. Panel (A) rCry11Aa Western Blot. Lane 1: Protein marker Bio-Rad Dual colour; Lane 2: Recombinant Cry11Aa toxin expressed in *Escherichia coli* BL 21 (DE3) C43 probed with anti-histidine antibody (~72 kDa); Lane 3: Negative control (BSA 10%); Panel (B) 12% SDS–PAGE of bacterial suspensions. Lane 1: Protein marker Bio-Rad Dual colour; Lane 2: recombinant Cry11Aa toxin expressed in *E. coli* BL21 (DE3) C43; Lane 3: *E. coli* BL21 (DE3) C43 control; Lane 4: *B. thuringiensis* var. *israelensis* spore suspension with protein profile ranging from 10 to 120 kDa; Lane 5: BSA at a concentration of 0·25 μg μL^{-1}; Line 6: BSA at a concentration 0·5 μg μL^{-1}; Lane 7: BSA at a concentration of 1 μg μL^{-1}.

Larvicidal effect of Cry11Aa

In vitro larvicidal effects against *Haemonchus* L2/L3 larvae were observed with both the native Cry11Aa expressed by *B. thuringiensis* and its recombinant form expressed in *E. coli*. We observed a larvicidal effect *in vitro* ($P < 0{\cdot}001$) of 62 and 81%, respectively, compared with the control group, for the native Cry11Aa expressed in *B. thuringiensis* and the recombinant Cry11Aa expressed in the *E. coli* suspension when added to feces that were naturally infected with *H. contortus*. Notably, the recombinant Cry11Aa expressed as an inclusion body in *E. coli* expressing ~1·2 μg μL^{-1}, had a significantly superior larvicidal effect of ~19% ($P < 0{\cdot}05$) more than the native toxin present in the crystal/spore suspension of *B. thuringiensis* that expressed an approximately 3·9 μg μL^{-1} (Fig. 2). Additionally, there was no difference in egg counting among the groups.

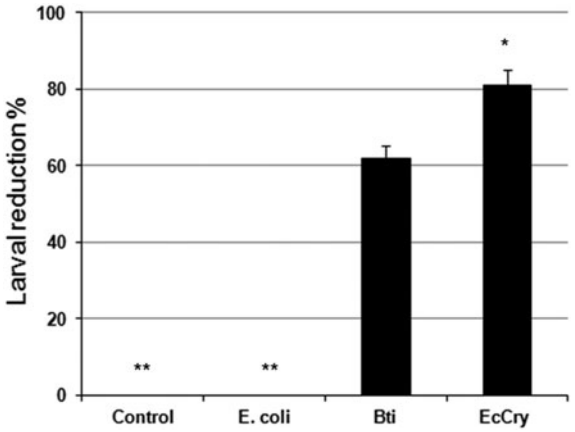

Fig. 2. Larvicidal effect of Cry11Aa *in vitro*. The data represent the mean (±S.D.) % larval reduction from three independent experiments, where *B. thuringiensis* var. *israelensis* (Bti, crystal/spore suspension 10^8 CFU mL^{-1}), recombinant *E. coli* expressing Cry11Aa (EcCry, suspension 10^8 CFU mL^{-1}), a non-recombinant *E. coli* (*E. coli* BL 21 (DE3) C43, suspension 10^8 CFU mL^{-1}) and control (H_20). Asterisks (**), represents statistical differences ($P < 0{\cdot}001$) among the Control, *E. coli* control and Bti, and EcCry groups, where (*) represent statistical differences ($P < 0{\cdot}05$) between Bti and EcCry groups.

Administration of B. thuringiensis *and recombinant* E. coli *expressing Cry11Aa to sheep naturally infected with* H. contortus

We observed that when the suspensions of *B. thuringiensis* and *E. coli* expressing recombinant Cry11Aa were administered orally to sheep naturally infected with *H. contortus*, both treatments demonstrated significant larvicidal effects ($P < 0{\cdot}001$) compared with the control group, thus reducing the percentage of viable larvae. The native *B. thuringiensis* Cry11Aa reduced the viable larvae by ~79%, whereas the recombinant *E. coli* expressing Cry11Aa reached a ~90% reduction of viable larvae compared with the control group (Table 1).

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Table 1. *Haemonchus* larvae reduction. Range and arithmetic average of L2/L3 larvae was counted, and the efficiency of the treatment was calculated

Treatments	EPG B	EPG A	Range	AM (±S.D.)	% Reduction
Control	2070 (±21·7)	2100 (±21·1)	(2000–2190)	2005·2 (±83·4)	–
Bacillus thuringiensis	2450 (±22·9)	2400 (±22·9)	(547–648)	420·4 (±46·3)	79·03%
Escherichia coli Cry11Aa	1800 (±12·9)	2000 (±17·1)	(160–266)	182·9 (±44·7)	90·87%
Escherichia coli Control	2130 (±18·3)	2020 (±19·2)	(2008–2219)	2010·7 (±87·5)	–

EPG B, mean eggs counts before treatments; EPG A, mean eggs counts after treatments (±S.D.); AM, Arithmetic means. The efficiency (% Reduction) was calculated using the formula $R = 100\ (1-T/C)$, where R is the reduction (larvicidal effect), C is the number of larvae in the control, and T is the number of larvae in the bacteria treated group.

DISCUSSION

Gastrointestinal parasites are a major cause of direct and indirect losses in sheep livestock. Different chemical drugs are increasingly being used in an ineffective endeavour to control these parasites, which have developed multidrug resistances to these traditional molecules (Kaplan, 2004; Geary *et al*. 2010; Medeiros *et al*. 2014). To circumvent the emergence of resistance, studies have been conducted in an attempt to decontaminate the grazing environment (Barger, 1999). Promising alternative options have included intercropping (rotational grazing of two or more different animal species) and recently, the potential use of biological control agents such as fungi (i.e. *Duddingtonia flagrans*) (Chandrawathani *et al*. 2002; Silva *et al*. 2015). In agriculture, biological control has been used for decades either in combination with or as an alternative to traditional pesticides (Betz *et al*. 2000). However, the potential for using this type of control against gastrointestinal parasites in livestock is still being investigated. In this study, direct evidence is given for the use of the Cry11Aa toxin as a biological control strategy for haemonchosis in sheep.

Our previous findings suggested that *B. thuringiensis* has the potential to be used as a biological anthelmintic in sheep flocks. Recently, our group reported on the larvicidal effect of different strains of *Bacillus* against *H. contortus* larvae in naturally infected sheep feces (Sinott *et al*. 2012). However, the *B. thuringiensis* strain used in this study possesses more than one toxin (Fig. 1B) within the range of 10–120 kDa, which suggests its larvicidal effect is due to one or more toxins or their associations. There is reference (Bone *et al*. 1988) that mentions a possible ovicidal effect by the *B. thuringiensis* toxins. However, like Wei *et al*. (2003) we did not observe any toxic effect in the egg hatching. Since the eggshell of nematodes are very resistant is unlike that the Cry11Aa can penetrated, so the effect observed by Bone *et al*. (1988), probably was mediated by non-Cry toxins.

In the present study, we observed that the principal larvicidal effect was mediated by the Cry11Aa toxin. Having been cloned and expressed in *E. coli*, it demonstrated an *in vitro* and *in vivo* larvicidal effect similar to, and even higher than the native *B. thuringiensis* crystal/spore suspension (Fig. 2 and Table 1).

Interestingly, when comparing the larvicidal effects using the same concentrations of microorganisms (2×10^8 CFU mL^{-1}), the *E. coli* expressing Cry11Aa was significantly more toxic both *in vitro* and *in vivo* against *Haemonchus* larvae than the *B. thuringiensis* crystal/spore suspension. The larvicidal effect of the recombinant *E. coli* was higher than that of the *B. thuringiensis*, showing larval reductions of 81% in the *in vitro* assays and 90% in the *in vivo* assays, which was approximately 19% higher than the *B. thuringiensis* suspension in the *in vitro* and approximately 11% *in vivo* assay. It was more surprising that *B. thuringiensis* expressed a higher toxin concentration than *E. coli* ~3·9 and 1·2 $\mu g\ \mu L^{-1}$, respectively, at the same bacterial concentration (Fig. 1B). In the free-living nematode, *Caenorhabditis elegans* has been suggested that this effect may be due to a defence mechanism that the nematode uses against several pathogens, thus preferentially moving towards an *E. coli* rather than a *Bacillus* and by doing so ingesting more *E. coli* than *B. thuringiensis* (Zhang *et al*. 2005; Hasshoff *et al*. 2007; Schulenburg and Ewbank, 2007). Also the toxin expressed as an inclusion body in *E. coli* might be easily solubilized in the larvae gut and by doing so might become more active (Höss *et al*. 2013).

Wei *et al*. (2003) found a 50% reduction in brood size at considerably lower concentrations of Cry $5B_2$ (IC50: 0·00047 mM) when the protein was supplied by *E. coli* expressing it in intact cells. This suggests that the availability of the toxin at the intestinal receptors of the nematode may be higher when the protein is taken up as inclusions in *E. coli* cells (ingested as food) than as dissolved proteins. However, we did not pursue this hypothesis because it was not the objective of this study. Nevertheless, there are many reports showing that Cry toxin expressed in *E. coli* is more efficient than the native Cry toxin expressed in *Bacillus* (Capello *et al*. 2006; Höss *et al*. 2013).

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An interesting feature of the Cry toxins is its larvae target specificity (Bravo *et al*. 2007). Species specificity is largely determined by the binding of a specific toxin to surface proteins located on the microvilli of larvae mid-gut cells, where the toxic effects occur (de Maadg *et al*. 2003). Factors such as intestinal pH, microvilli protein composition, cell type, and larvae diet may also affect toxin activity (Soberón and Bravo, 2007). In this study, we demonstrated that *B. thuringiensis* Cry11Aa toxin acts against *Haemonchus* larvae when orally administered to sheep. Kotze *et al*. (2005) reported that with a crystal/spore suspension of *B. thuringiensis*, the toxicity to nematode larvae was reduced when exposed to an acidic (pH $\leqslant$3) environment, suggesting the necessity of some protection for the toxin. This might be important because the sheep abomasum has a pH of 2–3 and is rich in gastric proteases (Kotze *et al*. 2005). However, Lee *et al*. (2002) reported the recovery of *B. thuringiensis* from the feces of different herbivores, and the recovered bacteria consisted of spores present in the consumed feed, suggesting that ingested spores/crystals are delivered intact to the feces of a feeding animal. We also reported that larvicidal *Bacillus* can multiply in sheep feces (Sinott *et al*. 2012), and it can be detected in the feces of the animal 4 h after its oral administration (Sinott *et al*. 2014). In this study, we observed that orally administered native or recombinant Cry11Aa toxin that was delivered as an inclusion body in *E. coli* was efficient in the control of *Haemonchus* larvae, reaching a 90% reduction in larva survival. One might suggest that *E. coli* cells act as protectors of the recombinant Cry11Aa toxin, ensuring that little to no degradation (i.e. proteases, low pH, etc.) occurs in the ruminant stomach. This allows delivery of the toxin as an inclusion body, when it is ingested by *Haemonchus* larvae in the stool bulk. Other studies have similarly reported that when they are expressed in *E. coli*, recombinant Cry toxins are more toxic for the target parasite than the native toxin expressed as a crystal/spore suspension. Höss *et al*. (2013) observed that recombinant Cry5B as an inclusion body expressed in *E. coli* was more toxic to (or more available to intestinal receptors) of *C. elegans*, than native toxin as a crystal/spore suspension. Capello *et al*. (2006) investigated the toxic effects of Cry5B on *Ancylostoma ceylanicum in vitro* and found that it was highly toxic to early stage hookworm larvae; moreover, exposure of adult parasites to Cry5B was associated with a substantial reduction in adult female worm egg excretions.

Implementing biological parasite control programmes that ensure the health of both the livestock and the environment avoids the problems associated with parasitic resistance and ecological toxicity. Biological control using *B. thuringiensis* var. *israelensis* Cry11Aa may become an important tool for pasture decontamination because its larvicidal activity disrupts the nematode's life cycle.

In summary, the most important conclusion from our findings is that Cry11Aa from *B. thuringiensis* var. *israelensis* demonstrates a strong potential towards controlling an important nematode in veterinary medicine.

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