Intranasal Immunization of Lambs with Serine/Threonine Phosphatase 2A against Gastrointestinal Nematodes

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Seven 3-month-old, female, helmint-free lambs were immunized intranasally with three doses (1 mg total) of a recombinant part of the catalytic region of the serine/threonine phosphatase 2A (PP2Ar) (group 1 [G1]). In addition, four lambs were used as an adjuvant control group (G2), four as unimmunized, infected controls (G3), and four as unimmunized, uninfected controls (G4). Fifteen days after the last immunization, lambs from G1, G2, and G3 were challenged with 10,000 larval stage 3 (L3) organisms in a plurispecific nematode infection composed of ca. 40% Trichostrongylus colubriformis, 40% Haemonchus contortus, and 20% Teladorsagia circumcincta. All the lambs were clinically monitored throughout the experiment. Parasitological (fecal egg output and immunological response), biopathological (packed-cell volume and leukocyte and eosinophil counts), and zootechnical (live-weight gain) analyses were conducted. On day 105 of the experiment, all the animals were slaughtered and the adult worm population in their abomasum examined. Intranasal administration of PP2Ar with bacterial walls as an adjuvant elicited a strong immune response in the immunized lambs, as evidenced by their humoral immune response. Immunized animals and animals receiving the adjuvant shed significantly (P < 0.001) fewer numbers of parasites’ eggs in their feces. The immunization significantly reduced the helminth burden in the abomasum by the end of the experiment (>68%), protection being provided against both Haemonchus and Teladorsagia. Live-weight gain in the immunized lambs was similar to that in the uninfected controls versus the infected or adjuvanted animal groups. Our results suggest that heterologous immunization of ruminants by intranasal administration may be efficacious in the struggle to control gastrointestinal helminths in these livestock.

Trichostrongyloides is a worldwide parasitic disease affecting ruminants of all species. Infection by these helmintos provokes digestive disturbances such as loss of appetite, diarrhea, alterations in energy and protein metabolism, and anemia, accompanied in severe cases by hypoproteinemia and edema. The relative severity of the clinical signs and the outcome of the disease depend on the host species, the age of the animals, the parasite load, and the specific composition of the infection. In extensive grazing systems, the general rule is a situation of mixed infections. Control of the disease has been based almost exclusively on the use of anthelmintics, but their massive and indiscriminate use has led to the appearance of parasite isolates with resistance to most of the drugs currently in use (1). In certain livestock-raising areas, resistance levels have reached nearly 90% against some of the anthelminitics (2), implying that the average viable life of new anthelmintics is estimated to be around 10 years. Under these conditions, alternative control methods should be explored, among them immunoprophylaxis. Discovering a vaccine against helminth parasites, which affect domestic animals as well as humans, thus adding economic damage to human suffering, constitutes a major challenge for researchers. So far, vaccination against gastrointestinal nematodes in ruminants has yielded only limited success (3, 4).

The success of vaccination systems, measured in terms of the reduction and viability of the eggs excreted, yields values from 32% to 90% and a reduction in the adult population of up to 78%. Their efficacy is determined not only by the type of antigen used but also by the adjuvant and the way of administration. Greatest efficacy has been achieved with vaccines that use irradiated larvae (5), but the logistical problems involved in their production and administration (6) have led to a search for native antigens or recombinant ones (7). The latter are easier to produce and distribute, and many of them are proteases, cysteine proteases, metalloproteases, aspartyl proteases (PEPs), all proteins from the helminth intestine, and some with unknown biological functions (8–11). Due to the homology in their sequences, some of these antigens have proved to be effective against different nematode species (3, 12, 13). In addition, most of the adjuvants used are oily suspensions such as Freund’s adjuvant, Montanide, or Lipovant (14–16). Freund’s adjuvant induces potentially serious, adverse side effects, granulomes or ulcers at the inoculation site, and therefore formulations with immunoadjuvant activity have been sought (17). Other adjuvants, including plant saponins (18), nanocapsules such as ISCOMs (19, 20), and lipopolysaccharides (LPSs) from bacterial walls or bacterial ghosts have been described (21, 22).

The immunization route is another key factor to be borne in mind in the development of vaccines (23). Most vaccines are administered either intramuscularly or subcutaneously, although the administration of vaccines through the mucosa offers a number of major advantages, such as easy administration and the reduction of adverse side effects. Furthermore, the fact that many
pathogenic agents invade the host via mucosal barriers renders the activation of immunity in the mucosa an attractive subject for further study (24).

Mucosal immunization techniques have been assayed against intestinal helminthes and the larvae of tissue-dwelling helminthes (25). Recently, Solano-Parada et al. (2010) (20, 26) obtained promising results after intranasal immunization against experimental angiostrongylosis, caused by Angiostrongylus costaricensis, using a recombinant part of the catalytic region of serine/threonine phosphatase 2A (PP2Ar) with bacterial walls as an adjuvant. Serine/threonine protein phosphatase (PP2A) is an enzyme that catalyzes the elimination of phosphate groups from the phosphorylated proteins. It has been incriminated in many biochemical and cellular processes such as cell motility, embryogenesis, and differentiation (28–30) and is present in many nematode species (20, 31–34). These characteristics make the catalytic region of PP2 a good candidate for an antigen to be used in vaccines, especially against parasites that must undergo morphogenic processes in the definitive host before reaching maturity.

Our aim has been to explore the potential immunoprotective value of a recombinant heterologous PP2Ar from A. costaricensis against a challenge with a multispecific infection by trichostrongylids in ruminants, which was administered intranasally to activate the mucosa, using the bacterial walls of Escherichia coli as an innocuous adjuvant.

MATERIALS AND METHODS

Lambs and experimental design. Nineteen 3-month-old, female, helminth-free lambs (Manchego breed) were obtained from a local producer, kept in isolation pens in our facilities, and clinically monitored throughout the experiment. They were fed with commercial pelleted food (Superfeed, Spain), hay, and water ad libitum. The conditions were approved by the Madrid Veterinary Faculty Committee for Animal Experimentation. The lambs were distributed into 4 stratified groups according to their live weight. They were immunized intranasally either with three doses (1 mg) of recombinant peptide (G1, 7 animals) or with the adjuvant at fortnightly intervals as an adjuvant control (G2, 4 lambs). In addition, 4 lambs were kept as unimmunized, infected control (G3) and 4 as unimmunized, uninfected control (G4). Fifteen days after the last immunization, lambs from G1, G2, and G3 were challenged with 10,000 larval stage 3 organisms (L3) of a plurispecific nematode infection composed of ca. 40% Trichostrongylus colubriformis, 40% Haemonchus contortus, and 20% Teladorsagia circumcincta. An infective dose was prepared using L3 obtained by coproculture (26°C, 10 days, and >80% relative humidity) of monospecifically infected lambs kept in our department. H. contortus was obtained from Merck, Sharp & Dohme, Spain, in 1987 and maintained in our facilities by serial passage in donor lambs. T. colubriformis and Teladorsagia circumcincta were originally supplied by the Moredun Research Institute (Edinburgh, Scotland) and maintained by serial infection in our department. All the animals were weighed at the beginning of the experiment and each week thereafter, blood and serum samples being taken at the beginning, after immunization, before the challenge, and at slaughter, as described below (Fig. 1).

Antigen and adjuvant: recombinant protein. We used a recombinant peptide corresponding to the catalytic region of the serine/threonine protein phosphatase (PP2A) expressed by a CT2-2 clone corresponding to the PP2Ar catalytic region of A. costaricensis, produced as described elsewhere by Solano-Parada et al. (20). This fragment had the sequence VVDIICTTNNHINNLIDLRHAHQTATEMYGGYRYIFAGGLVTIFSPNYPQNMNMND GCVMRKIDLTANIFRFPVRRH. We began with the clone A TripIEx-2 -CT2-2, described elsewhere (20), and converted it to pTripIEx 2-CT2, which was sequenced with an ABI PrismTM BIGDYE sequencing kit (Applied Biosystems, Foster City, CA, USA) using the forward primer 5’TCC GAGATCTGGACGAGC3’ and the reverse primer 5’CCCTATAGTGAG TCATTTAA3’. The CT2-2 sequence was confirmed as corresponding to the catalytic region of gene pph-1 serine/threonine protein phosphatase (NCBI accession number CAJ18121.1 [23]). The CT2-2 sequence was cloned into the expression vector pET40a+ (Novagen), which was replaced by tRNAs for 7 codons rarely used in E. coli (AGA, AGG, AUA, CUA, GGA, CCC, and CGG) and enhanced the expression of the eukaryote proteins that contain these codons. The recombinant protein in the form of inclusion bodies was purified from colonies isolated in LB plates with ampicillin (100 μg/ml) and chloramphenicol (34 μg/ml) before being cultured for 12 h in 2× YT ampicillin-chloramphenicol broth. Recombinant production was induced with 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for 3 h and then centrifuged at 4,000 ×g for 10 min. The pellet was frozen at −20°C for at least 24 h, thawed in ice, and resuspended in lysis buffer containing 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 10 mM EDTA, 5 mM β-mercaptoethanol, 0.35 mg/l lysozyme, 8 U/ml Benzo nase (Novagen), and 0.5% Triton X-100 before being incubated for 30 min at 20°C. This was followed by sonication with 6 cycles of 10 s at 200 to 300 W. The lysate was centrifuged again at 10,000 ×g for 30 min at 4°C.

The protein pellet was obtained three times with phosphate-buffered saline (PBS) and resuspended in sterile distilled water. Inclusion bodies were lyophilized and solubilized in 20 mM sodium phosphate buffer containing 8 M urea, 0.5 M Na Cl, 20 mM imidazole, and 1 mM β-mercaptoethanol, pH 7. Purification of the purified protein was carried out by affinity chromatography with nickel-agarose, nickel-nitrilotriacetic acid (Ni-NTA) (Qiagen), previously equilibrated with 20 mM sodium phosphate, 0.5 M NaCl, and 20 mM imidazole, pH 7.4. The sample was loaded, and the column was washed with 20 mM sodium phosphate, 0.5 M NaCl, and increasing concentrations of imidazole, from 10 mM to 100 mM. A final elution was performed with 20 mM phosphate buffer with 8 M urea, 0.5 M Na Cl, 500 mM imidazole, and 1 mM β-mercaptoethanol, pH 7.4 (35). All fractions (uninduced, induced, purified, and nonpurified) were analyzed by 12.5% SDS-PAGE and stained with Coomassie brilliant blue dye (Fig. 2).

The recombinant protein was sequenced and identified at the Servicio de Proteómica del Centro de Biología Molecular Severo Ochoa (CBMSO) in Madrid, Spain. The relevant band from the SDS-PAGE was excised in situ with a robot digester (Bruker) using trypsin according to a protocol described elsewhere (36). The supernatant from the digestion (containing the peptides) was acidified with trifluoroacetic acid (final concentration, 0.1%) and dried in a Speed Vac (Thermo) before being resuspended in 0.1% trifluoroacetic acid with 33% acetonitrile. A 0.5-ml aliquot was placed on an AnchorChip plate (Bruker) using 2.5-dihydroxybenzoic acid (DHB) as a matrix, to a concentration of 5 g/liter via the “fast evaporation” method. The plate was measured in an Autoflex matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometer (Bruker) equipped with a reflector. The mass spectra thus obtained were used as a peptide fingerprint to identify proteins in the database using search engines available on the Internet (MASCOT or ProFound).

The protein concentration of the PP2Ar solubilized in denaturizing buffer (6 M urea) was determined spectrophotometrically at 595 nm with the Bio-Rad protein assay. After electrophoresis in SDS-PAGE (12%), the separated proteins were transferred to a nitrocellulose membrane (Hy-
FIG 2 SDS-PAGE analysis of purified PP2A. Lane 1, total proteins of the transformed bacteria; lane 2, PP2A band after affinity chromatography with nickel-agarose column purification; lane 3, recognition by the immune serum against the PP2Ar. Molecular mass is given in kDa.

FIG 3 Multiple alignments (ClustalW2) of the sequence of the catalytic center of PP2A in different nematodes. Black indicates the positions with 100% conservation, while gray represents a decline in conservation. A rectangle marks the sequence of PP2A from *Angiostrongylus costaricensis* (GenBank accession number AAO85518) (33), TcPP2A from *Trichinella spiralis* (NCBI accession number ABL14203) (39), BmPP2A from *Brugia malayi* (NCBI accession number XP_001892306) (31), and AsPP2A from *Ascaris suum* (NCBI accession number ADY46840) (40) were then aligned and edited using ClustalW with GeneDoc programs.

Blood sampling and parasitological, biopathological, and immunological determinations. During the experiment, the animals were daily observed for clinical monitoring and possible adverse reactions. Individual coprological analyses were carried out with a modified McMaster technique (41). Fecal egg output values were log transformed to normalize the values used for statistical and graphic representations. Throughout the experiment, blood samples were obtained by jugular venipuncture in evacuated tubes every 14 days. Packed-cell volume (PCV) and leukocyte and eosinophil counts were determined by standard laboratory techniques. Serum-specific antibody response was determined by enzyme-linked immunosorbent assay (ELISA). Briefly, MaxiSorpTM 96-well microplates (Nalge Nunc Intl., Rochester, NY, USA) were coated with 10 μg/well of PP2Ar. The antigens were diluted in 0.1 M NaHCO₃ (pH 8.6) to give a concentration of 10 μg/well. Individual lambs’ sera were diluted 1:100 to 1:1,600 in carbonate buffer at pH 8.6, and the secondary antibody was anti-sheep IgG (whole molecule) peroxidase conjugate (Sigma) diluted 1:10,000. Absorbance was read at 492 nm (Multiskan Spectrum; Thermo Scientific).

On day 105 postchallenge, the animals were slaughtered at a local abattoir (Getafe, Madrid), whereupon the abomasum and small intestines were removed and taken to the laboratory under refrigeration. Individual abomasum were opened, and the mucosas and adult helminths in the content were washed in cold PBS. A 10% aliquot of all the helminths recovered was fixed in 5% buffered formalin, and the worms present were counted (males and females) under a stereomicroscope (Leitz).

Statistical analysis. Logarithmic transformation of the titers in sera and egg numbers was used for the graph representation and statistical analysis. The Tukey-Kramer multiple-comparisons test was used to estimate the significance of the difference between means. The results are indicated as mean values (standard errors of the mean of the different groups at different times for each experiment performed were determined). A P value of <0.001 was considered to be highly significant, and a P value of <0.01 was considered significant. GraphPad Instat v3.05 (GraphPad Software, Inc., La Jolla, CA, USA) software was used for the statistical analysis.

RESULTS AND DISCUSSION

After analyzing the recombinant protein sequence obtained and carrying out a multiple alignment using the ClustalW program, we confirmed the high homology of the sequence with that from the catalytic region of serine/threonine phosphatase (PP2Ac) in other species of nematodes, including some species of interest in human and veterinary medicine, such as *Trichostrongylus vitrinus*, *Oesophagostomum dentatum*, *Trichinella spiralis*, *Brugia malayi*, and *Ascaris suum* (Fig. 3). The catalytic subunits of PP2Ar (36 kDa) and PP2A (65 kDa) constitute the constant structural sub-
units of the enzyme core. Both subunits have only two isoforms, but the third subunit, known as subunit b, has numerous families, each of which in turn has multiple isoforms (28, 29, 42). PP2A is ubiquitous, structurally conserved, and involved in many cell processes (43), including the functioning of the cytoskeleton (28), flagellum mobility (35), and cell cycle and meiosis (44). Götz et al. (45) have also suggested its involvement in embryonic development. Furthermore, it participates in the mechanisms of cell signaling (29, 46) and its deregulation gives rise to pathological processes such as cancer (43, 47–49).

The intranasal administration of a potential immunogenic molecule with bacterial cell walls of *E. coli* was very effective under our conditions since all the lambs in G1 showed significantly high levels of IgG anti-PP2Ar after day 28 (before the second vaccination set) until the end of the experiment (Fig. 4).

Intranasal immunization has been used before both in laboratory mouse models (24, 50, 51) and in domestic animal species (52), while bacterial components have also been used successfully in immunization (21, 22, 53, 54), but the combination of these methods for nematode vaccination has not been tested except in a recent experiment using the same protein, PP2Ar, against *A. costaricensis* (20) in mice. Immunization provoked lower parasite burdens and increased levels of interleukin-17 (IL-17) and specific IgA. Recently, the synthesis of specific IgA has been found to be dependent on the Th17 response by Peyer’s patches in the intestine (55, 56).

Our results confirmed the validity of this method of immunization and its potential use in ruminants. Moreover, the higher antibody levels observed in vaccinated lambs could indicate a massive liberation of membrane and intracellular antigen resulting from the death of the helminthes. The relatively low titers found in the infected and adjuvanted group may indicate that the native antigen is not an excreted antigen by the worms.

No clinical signs were observed in any of the challenged animals throughout the experimental period. The hematological parameters determined are shown in Fig. 5. PCV diminished slightly in all the groups throughout the experiment (Fig. 5, top left), although the reduction was within the physiological range and unrelated to the infection status of the lambs. The lack of any significant variation in the unimmunized, infected lambs (G2) could be due to the relatively moderate burden of *H. contortus* in the infective dose. Similarly, leukocyte counts in peripheral blood did not show any variation (Fig. 5, top right). However, eosinophils displayed both infection- and immunization-related behavior (Fig. 5, bottom). In spite of the wide variations found, the immunized lambs had significantly higher levels than all the other groups on days 70 (G2 and G3, *P* < 0.05; G1, *P* < 0.01), 84 (*P* < 0.05), and 97 (G3 and G4, *P* < 0.05; G2, *P* < 0.01) postimmunization. A rise in eosinophil counts is considered to be a distinct characteristic of helminth infection (57), and eosinophilia has in fact been related to the protection of lambs (58–60), although this response is variable (61). The absence of clinical signs and notable hematological variations could be related to the moderate infective dose administered.

Figure 6 shows the results of the fecal egg output along the patent period of the infection. Unvaccinated and challenged groups (groups 2 and 3) showed an increase in egg values along the experimental period, whereas vaccinated lambs displayed a plateau 8 weeks after challenge onwards. Significantly higher egg values were found in the nonvaccinated animals. However, as expected, there was a high variability among individual lambs from each group. This finding is frequently observed in lambs given a primary infection with gastrointestinal helminths, both in naturally infected animals with plurispecific infections and in controlled experiments with single-species administration (9). It has been considered the expression of the “responder” and “nonresponder” animal phenotype described in this host parasite system with *T. colubriformis* (62) and *H. contortus* (63). In turn, this intragroup variability makes necessary the transformation of egg values for statistical analysis (64). In our experiment, the infective dose was composed of three species from the genera *Haemonchus*, *Teladorsagia*, and *Trichostrongylus*. The reproductive capacity of *H. contortus* is much higher than that of the other two genera employed, and therefore, fecal egg output is a poor estimation of induced protection when plurispecific infections are employed. Individual coproculures were carried out only at the end of the experiment (day 105 postinfection [p.i.]). *H. contortus* third-stage larvae were the most commonly recovered, and only immunized lambs showed a significant (*P* < 0.05) reduction of *Trichostrongylus* L3 obtained from the eggs compared to the adjuvant control and unimmunized challenged groups (G2 and G3) (not shown).

More relevant were the results of helmintdf burden and live-weight gain. Adult burden provides a good estimation of induced protection and has been extensively used in immunization trials against gastrointestinal (GI) helminths. As expected, the recovery of *Trichostrongylus* from the small intestine yielded inconsistent results, which were excluded from further analysis. The specific composition of the adult helminths in the abomasum of challenged lambs varied widely. No absolute establishment rate (ER) could be determined since all the lambs were slaughtered at the end of the experimental period, and the possibility of some of them having expelled part of the infective dose cannot be ruled out. The ER found for *H. contortus* in the unimmunized challenged animals was comparable to that found for other isolates (65, 66) and sim-
ilar to results obtained previously with this parasite stock (67), and thus no conclusion could be reached. The most abundant species in the three infected groups was *Teladorsagia circumcincta* (about 3 times more abundant than *Haemonchus*) in spite of the lower number of infective larvae administered. One lamb (lamb 4) from the immunized group (G1) and another from the unvaccinated challenged group (G2) (lamb 9) did not show any *Haemonchus* in their abomas.

Under our conditions, immunization with PP2Ar led to a reduction in adult helminth burdens in the abomasum by the end of the experiment. A protective effect ($P < 0.05$) was observed against the total populations of *H. contortus* (78.33% reduction) and *Teladorsagia circumcincta* (68.56% reduction) compared to unimmunized challenged lambs (Fig. 7). Notably, these protection levels were achieved against two species of nematodes with different feeding behaviors. Only lambs from G1, receiving bacterial walls plus PP2Ar, displayed any significant reduction in the

![FIG 5](image-url) Variation in physiological parameters determined in the lambs throughout the experiment: packed-cell volume (PCV) (top left); leukocytes (top right); and eosinophils (bottom). Values were determined in peripheral blood. Symbols in top panels: +, vaccinated; ○, unimmunized and challenged; ▲, adjuvant control group; ▼, unchallenged control animals. Code for bottom panel: black column, immunized; gray column, unimmunized and challenged; hatched column, adjuvant control group; clear gray column, uninfected control animals. For all panels, the arrow shows the day of challenge. Values are means ± standard deviations.

![FIG 6](image-url) Fecal egg output (means ± standard errors) throughout the patent period. Individual egg counts were log transformed. **, significant differences ($P < 0.01$); ***, highly significant differences ($P < 0.001$). White, vaccinated group (G1); gray, unimmunized and challenged group (G3); dark gray, adjuvant control group (G2).

![FIG 7](image-url) Adult helminth burden (means ± standard deviations) from the abomasum of experimental lambs. Numbers of adult worms from all the lambs were determined at the end of the experiment. Light gray, vaccinated animals; gray, nonvaccinated, nonadjuvanted animals; dark gray, adjuvanted animals. *Haemonchus, Haemonchus contortus; Teladorsagia, Teladorsagia circumcincta; F, female; M, male; ns, not significant. Values are means ± standard deviations.
adult burden of *Haemonchus* and *Teladorsagia*. However, the administration of the adjuvant apparently elicited some degree of protection against challenge since the parasite burdens in this group (G3), although not significant, showed consistently lower counts than unimmunized challenged lambs (G2). The immunomodulatory and immunostimulant properties of bacterial walls have been described (21, 22, 53). In addition, exposure to lipopolysaccharides (LPSs) from bacterial walls induces the production of nitrogen species and reactive oxygen as well as proinflammatory cytokines by macrophages (68). Since the need for stimulation of mucosal response to induce protection in helmint infections has been suggested, it is possible that the bacterial walls elicited an unspecific activation of mucosal immunity in our experiment. Some genes associated with the early inflammatory response, including those encoding toll-like receptors (TLR2, -4, and -9) or involved with free radical production (DUOX1 and NOS2 A), are more abundantly expressed in lambs that are resistant to *H. contortus* and *Trichostrongylus colubriformis* infections (69). Further experimentation is needed, but this activation could be responsible for the apparent reduction of helmint burdens in nonvaccinated lambs receiving the adjuvant (G3).

Live-weight gain in lambs is an important zootechnical parameter of the “resistant” or “resilient” status of animals. Figure 8 shows the live-weight gain in the four groups of lambs. Overall, analysis showed that the immunized and uninfected control lambs maintained similar weight gains throughout the experiment. Nevertheless, infection led to weight loss in unimmunized lambs 6 weeks p.i. compared to the uninfected controls (*P* < 0.05). At 8 weeks p.i., the immunized lambs (G1) showed no difference from the control group (G4) whereas the unimmunized ones (G2) were significantly lighter (*P* < 0.05). This finding is corroborated by the fact that no significant differences were found during week 0 of infection and preinfection (week −8) (not shown).

Vaccination of ruminants against GI helmints, particularly trichostrongylids, has proved to be an elusive issue for decades (4). Thus, in spite of numerous attempts with both “hidden” and “exposed” antigens (12, 70, 71), there is no commercially available vaccine against *Haemonchus*. Partial protection induced by native forms of parasite proteins has not yielded results comparable to those of recombinant products. Failures have been related to the presence of nonresponder sheep and inappropriate glycosylation or folding of recombinant proteins, besides our poor knowledge of the relevant mechanisms of the sheep immune system (71).

With regard to other important gastric helmints infecting small ruminants, both in terms of pathology and economics, the identification of native protective antigens is probably lacking (72). At present, whereas conventional immunization procedures work for *Ostertagia* in cattle, they are unable to elicit protective responses against the closely related genera *Teladorsagia* and *Trichostrongylus* in sheep and goats (73).

Our results showed that PP2Ar administered intranasally with *E. coli* walls can elicit a partially protective response against *H. contortus* and *Teladorsagia* *circularis* in lambs, reflected in the similar weight gains of immunized animals and the notable reduction in the plurispecific helmint burden in the abomasum (over 68% at least). This protection, achieved with a recombinant heterologous protein, is related to mucosal immunization with the adjuvant and the antigen (PP2Ar) and is expressed against two different species of gastrointestinal nematodes. Anthelmintic resistance is a widespread phenomenon (2, 74–76), and the immune protection elicited would reduce the need to medicate animals in risk areas and seasons. As such, effector mechanisms, in particular the role played by the bacterial adjuvant employed, should be explored, but our results with the intranasal route and the possibility of heterologous immunization against plurispecific helmint infections are encouraging.

**ACKNOWLEDGMENTS**

This research was financed by a Spanish Ministry of Science and Technology grant (AGL2011-26098) and the “Programa de Ayudas para la Transferencia de Resultados de Investigación” of the OTRI, University of Granada, Spain.

We thank B. Rojas for her technical assistance. We also thank J. Trout of the Scientific Translation Service of the University of Granada for revising and editing the English text.

The patent protecting these results is licensed exclusively to Bioorganic Research and Services SA.

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