Manuscript Details

Manuscript number	*PARINT_2016_208*
Title	*Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: humoral response to the vaccine*
Article type	*Research paper*

Abstract

Cystic echinococcosis is endemic in the Rio Negro province of Argentina and, for this, a control program using praziquantel in dogs was developed from 1980. The transmission rate to humans and sheep has decreased significantly, however transmission persists. In 2009 the vaccination of sheep with EG95 was incorporated in some areas of the province. The objective of the study was to evaluate the humoral responses to the vaccine EG95. Lambs received two vaccinations with the EG95 vaccine followed by a single booster injection when the animals were 1-1.5 years of age. Blood samples from 6 vaccinated groups and 4 no vaccinated sheep were obtained for determination of antibody titles against EG95 protein. Anti-EG95 responses were determined as described by Heath and Koolaard, 2012. Responses were evaluated from 331 animals. Median ELISA absorbance values in vaccinated group was 0.828, and in non-vaccinated groups was 0.218. EG95 antibody responses in sheep from different cohorts of non-immunized control groups and vaccinated groups reveal the sustained increase in response seen in animals following the third immunization. ($p< 0.0001$). Significant differences are also evidenced in ANOVA test ($p< 0.001$). An anti-EG95 antibody response was induced in all groups of immunized sheep, 4 times higher (0.828) than the mean observed in the control groups (0.218). Data described here indicate that following a third vaccination with the EG95 vaccine at 1 year of age the specific IgG responses detected in the serum of sheep increased to a level greater than that seen following the second immunization and that this response was maintained longitudinally over time, for at least 5 years

Keywords	*echinococcosis; vaccine EG95; sheep; humoral response*

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Submission Files Included in this PDF

File Name [File Type]

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vaccinated area EG95.kml [Interactive Map Data (.kml, .kmz)]

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Prof Hoffmann
Editor
Parasitology International
Present

PARINT_2016_208R2

Dear Karl,

I am sending our paper “Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: humoral response to the vaccine” by your revaluation. We expect a positive response for a hard fieldwork in the Patagonia Region in Argentine.

Your request for English revision of this manuscript was unnecessarily derisory in my opinion. I had edited this manuscript extensively prior to its submission to you. It was unreasonable of you to characterise the submission as having mistakes in grammar, spelling and word usage throughout. Indeed I found few such errors when I now reviewed the manuscript for re-submission.

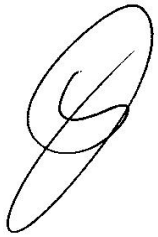
This manuscript was written by a first author for whom English is not their first language. I am co-author on many papers written by other authors whose first language is not English. In these situations, I edit the grammar and spelling for correctness but only edit expression in situations where the language used did not adequately convey the appropriate meaning. I do not re-write the whole manuscript in these situations. From a scientific publication point of view, so long as the meaning is clear and understandable, and the English expression is not technically incorrect, an author’s choice of expression is a reflection of their personality and should not need to be homogenised to some bland form of commonality.

Sincerely,

Marshall Lightowlers

Apologies for the mistakes.

Yours truly



Edmundo Larrieu

Prof. Dr. UNLPAM

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Title: Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: humoral response to the vaccine

Journal: Parasitology International

Dear Reviews

We have made the following changes to the manuscript :

There are some suggested alterations to the Summary, *We have made changes in the Summary:*

Cystic echinococcosis is endemic in the Rio Negro province of Argentina and, for this, a control program using praziquantel in dogs was developed from 1980. The transmission rate to humans and sheep has decreased significantly, however transmission persists. In 2009 the vaccination of sheep with EG95 was incorporated in some areas of the province. The objective of the study was to evaluate the humoral responses to the vaccine EG95. Lambs received two vaccinations with the EG95 vaccine followed by a single booster injection when the animals were 1-1.5 years of age. Blood samples from 6 vaccinated groups and 4 no vaccinated sheep were obtained for determination of antibody titles against EG95 protein. Anti-EG95 responses were determined as described by Heath and Koolaard, 2012. Responses were evaluated from 331 animals. Median ELISA absorbance values in vaccinated group was 0.828, and in non-vaccinated groups was 0.218. EG95 antibody responses in sheep from different cohorts of non-immunized control groups and vaccinated groups reveal the sustained increase in response seen in animals following the third immunization. ($p < 0.0001$). Significant differences are also evidenced in ANOVA test ($p < 0.001$). An anti-EG95 antibody response was induced in all groups of immunized sheep, 4 times higher (0.828) than the median observed in the control groups (0.218). Data described here indicate that following a third vaccination with the EG95 vaccine at 1 year of age the specific IgG responses detected in the serum of sheep increased to a level greater than that seen following the second immunization and that this response was maintained longitudinally over time, for at least 5 years

L 11- taeniacidal; *changes for taeniacidal drug*

Sentence in lines 19-21 incorrect., L 20- have =who; Sentence construction mistage in line 24.

We changes paragraph.

Historically CE was a highly endemic disease in the Rio Negro province of Argentina. CE control program was launched in 1980 using the existing primary health care infrastructure to deworm dogs. A group of health care assistants (nonprofessional staff) conducted home visits, while veterinarians from the health department lent support and managed the surveillance system. This network carried out four rounds of home visits annually. The health care assistants visited rural areas distributing praziquantel tablets to dog owners who were ultimately responsible for carrying out the deworming

L 27-sufficiently; *We changes paragraph*.
The Rio Negro program, however, incorporated additional surveillance methods for the human population. These included serological (initially the DD5 diffusion test, then from 1993 the ELISA) and from 1997, abdominal ultrasound surveys for the 6–13 years age group. The program has been successful in reducing the incidence of CE in humans (5.6% to 0.3% in school children) and dogs (41.5% to 2.5%), but not sufficiently to prevent continued transmission of the parasite and the continued incidence of human disease

L 35- induced by oncospheral antibodies?= acts against the invading *oncosphere; .We changed paragraph*.
Evidence from the transfer of vaccine-induced immunity with serum or colostral antibodies indicates that antibodies play a significant role in immunity to taeniid cestodes that is induced by anti-oncospheral antibodies

line 44: Sentence/wording mistake. *We changed paragraph*.
The control program in Río Negro decided the introduction of the vaccine as an additional control tool in some areas of the province. Vaccination program began in December 2009

lines 50 and 51: "positive" for what?
Twelve of the 154 vaccinated animals were determined to be positive to E granulosus

lines 57-58: I cannot understand this sentence at all. *We have rewritten paragraph*.
this trial has demonstrated the EG95 vaccine is a valuable tool to assist with reducing E. granulosus transmission, even in circumstances where delivery of the program faces many practical difficulties

lines 60-62: Sentence needs to be rewritten . *We have rewritten paragraph*
There is little information of the humoral response and the longevity of immunity against EG in sheep induced by EG95 vaccine [12]. Therefore, the objective of this study was to evaluate the humoral response and its evolution over time in sheep vaccinated with EG95 under field conditions in the Rio Negro control program

81-84: Sentence/wording mistake. .*We writing the paragraph*.
The EG95 vaccine used was produced by the University of Melbourne [7]. The vaccine was lyophilized and provided in vials containing 50 or 100 doses. The vaccine was rehydrated with sterile distilled water on the morning of the day of use. One ml of reconstituted vaccine containing 50µg EG95 and 1 mg Quil A [13,14].

Lines 138-139 and then 153: complete confusion regarding "mean", "median" and "average" ! .. needs major reconsideration. *We rewriting the paragraph and changes de figure.*
Median ELISA EG95 absorbance values and interquartile range from of vaccinated (0.828, 0.530) and non-vaccinated (0.218, 0.138) groups of sheep are shown in Figure 1. Student t-test analyses indicate significant differences between OD_{405nm} values from EG95 immunized and control groups of sheep ($p< 0.0001$). Significant differences are also evidenced in ANOVA analyses which indicate significant differences in the OD_{405nm} values when 4 cohorts of non- vaccinated animals and 6 cohorts of vaccinated sheep ($F= 75.5$, $p<0.001$, R^2 67.3) were compared. ANOVA also demonstrated significant differences in the OD_{405nm} values when only the 6 cohorts of vaccinated sheep ($F=17.8$, $p<0.001$) were considered.

Line 152: which ELISA? specify if it is EG95-ELISA. *Corrected*.
Mean ELISA EG95 absorbance

L 174-shown; are shown

L 184- YT group?; *we changes paragraph.*
Protection against infection afforded by the vaccination has been detailed by Larrieu et al. [16]. Four sheep in yellow tag (YT) group were…

L 262 ultrasonographic; ultrasonographic screening …

L 289- reference has no title. *We put the title***.**
Serological monitoring of protection of sheep against *Echinococcus granulosus* induced by the EG95 vaccine.

Figure 2 Explain NE and RN. *We change the figure*. RN = Rio Negro. NE not endemic area

A further comment on Figure 2. It appears that there were some old animals, presumeably vaccinated 3 times, that had lost their anti-EG95 antibodies. Could these have been those that missed out on most of the vaccinations? You state in the Discussion that often animals are not presented for the herd vaccination, for various reasons, and that giving 3 injections

would help to stimulate antibody in those who might miss one of the 3. However, a total negative response is unlikely and the reason for this should receive some discussion.
and a call for more discussion on the variability of serological responses in the 5 year-old group

We added paragraphs in the in lines 221-233

The observation of major variability of OD value between the dataset of vaccinated sheep compared with non-vaccinated control groups, which show lower variability, can be produced by the different individual response to the vaccine. Too, the native communities where this control program was undertaken are remote and have rudimentary infrastructure. In many instances, it was also not possible for the farmers to have all their animals available for vaccinations when they were due. For this reason, some animals may have missed one or more of their scheduled vaccinations. Unreliability in being able to deliver immunizations to individual animals is likely to have contributed to the variability in individual antibody responses observed and likely also contributed in the lower level of protection observed in the field trial compared to the results following experimental challenge infection in vaccinated sheep [12]. This trial was undertaken using procedures that would be expected to apply if EG95 vaccination were implemented as a routine procedure for the on-going prevention of E. granulosus transmission. In such circumstances, animals would not be expected to be identifiable individually, and some animals may fail to be mustered and miss one or more of their scheduled animal health treatments, eg vaccinations.

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1. Introduction

Cystic echinococcosis (CE) is a parasitic disease caused by infection with the larval stage of the cestode parasite *Echinococcus granulosus* (EG). The infection is commonly transmitted between livestock animals, especially sheep and goats, and dogs. Humans may also develop CE if eggs from the faeces of an infected dog are accidentally ingested. In humans, the infection manifests as cystic masses, most commonly in the liver and/or lungs. The disease is recognized by the World Health Organization as an important Neglected Tropical Disease [1,2]

Since the development of the highly effective taenicidal drug praziquantel, globally most efforts to control transmission of *E. granulosus* have relied on treatment of dogs with this drug. However, in many areas, difficulties accessing the dog population on a sufficiently regular basis have limited the effectiveness of control efforts, such that interruption of the parasite's transmission has not been achieved [1,3].

Historically CE was a highly endemic disease in the Rio Negro province of Argentina. A control program for CE was launched in 1980 using the existing primary health care infrastructure to deworm dogs. A group of health care assistants (non-professional staff) conducted home visits, while veterinarians from the health department lent support and managed the surveillance system. This network carried out four rounds of home visits annually. The health care assistants visited rural areas distributing praziquantel tablets to dog owners who were ultimately responsible for carrying out the deworming [4].

The Rio Negro program, however, incorporated additional surveillance methods for the human population. These included serological studies (initially the DD5 diffusion test, then from 1993 the ELISA) and from 1997, abdominal ultrasound surveys for the 6–13 years age group. The program has been successful in reducing the incidence of CE in humans (5.6% to 0.3% in school children) and dogs (41.5% to 2.5%), but not sufficiently to prevent continued transmission of the parasite and the continued incidence of human disease [1,5,6].

The EG95 vaccine for livestock animals has been developed as a new tool to assist in the control of *E. granulosus* transmission. The vaccine is highly effective in reducing CE in sheep exposed to an experimental infection with *E. granulosus* [7-10]

Evidence from the transfer of vaccine-induced immunity with serum or colostral antibodies indicates that antibodies play a significant role in immunity to taeniid cestodes that is induced by vaccination [10]. Further evidence indicating a role for complement-fixing antibodies in immunity stimulated by recombinant oncosphere antigen vaccines, including EG95, comes from in vitro oncosphere killing assays in which parasites are killed in culture in the presence of serum from vaccinated animals. A clear association has been found between the presence of specific antibodies induced by the EG95 vaccine and protection against infection in sheep [11,12]. Little data are available about use of the EG95 vaccine in field situations and the induction of protective levels of antibody by EG95 vaccination.

The control program in Río Negro decided to introduce the vaccine as an additional control tool in some areas of the province. The vaccination program began in December 2009.

The first evaluation of the impact of EG95 vaccination was undertaken using serological methods in 2012. That initial assessment was made in 275 two-year-old sheep based on ELISA/WB. Twelve of the 154 vaccinated animals were determined to be positive for *E. granulosus* infection (7.8%) while in the control area 33 out of 84 sheep were found positive (39.3%), $p<0.05$ [13]. A second evaluation of impact was made using necropsy in old sheep in 2015. Vaccinated sheep had a significantly decreased prevalence of *E. granulosus* infection in adult animals, 21.1% in 2015 compared to 56.3% in 2009 (P=0.03). In relation to the number and size of the hydatid cysts, 1.5 cysts per animal were found in the control area whereas 0.3 cysts were found per infected animal in the vaccinated area after 5 years of the program [14]. This trial demonstrated that the EG95 vaccine is a valuable tool to assist with reducing *E. granulosus* transmission, even in circumstances where delivery of the program faces many practical difficulties.

There is little information of the humoral response and the longevity of immunity against EG in sheep induced by EG95 vaccine [12]. Therefore, the objective of this study was to evaluate the humoral response and its evolution over time in sheep vaccinated with EG95 under field conditions in the Rio Negro control program.

2. Materials and Methods

The regions chosen for the vaccination program were Anecon Grande, Rio Chico Abajo, Mamuel Choique and Nahuel Pan and the control regions without vaccination were Blancura Centro and Lipetren (Latitude and longitude Anecon Grande -41.3215 -70.2742, Rio Chico abajo -41.7098 -70.4761 and −40.4238 −69.6146 Blancura Centro). The geographic region was the Rio Negro Province in Argentina comprising in total an area of 5820 Km2.

Among the selected communities there are five health centres, each employing a sanitary agent responsible for the first contact of the centre with the farmers. In these areas, at the start of the program 16511 sheep and 4696 lambs were present in the trial regions, of which 9383 sheep and 3146 lambs were in the vaccination area and 7128 sheep and 1550 lamb were in a control area, where there was a range of 10 to 200 animals per producer. It is common that land is not subdivided with fences, resulting in trans-boundary movement of sheep and dogs.

The EG95 vaccine used was produced by the University of Melbourne [7]. The vaccine was lyophilized and provided in vials containing 50 or 100 doses. The vaccine was rehydrated with sterile distilled water on the morning of the day of use. One ml of reconstituted vaccine contained 50µg EG95 and 1 mg Quil A [13,14].

All the vaccinated animals were ear tagged using a different colour tag for each year of the project: yellow (YT) in the first year and successively white (WT), red (TR), green (GT), blue (BUT) and black (BKT). The animals were not individually identified, therefore, an animal with tag is an animal that received at least one dose of vaccine (Table 1). The vaccination schedule was two doses of

97 vaccine in young lambs at approximately a 30 days' interval plus a single
98 booster injection given when the animals were one year of age.

99 Over the 6-year course of the study, the number of vaccine doses
100 employed was 21447 in six yearly cohorts of lambs. Of these, 6431 were in
101 lambs with a yellow tag (YT) in the first year, and successively 4449 in lambs
102 with white tag (WT), 1949 red tag (TR), 2562 green tag (GT), 3001 blue tag
103 (BUT) and 2865 black tag (BKT) tag in the last year (16). During the third year
104 of the programme the eruption of the Puyehue Volcano in Chile, affected the
105 programme. Ash falling over the work area led to the death of many animals.

106 In the 6th year of the program, in the vaccination region in each farm 2
107 sheep were selected of each colour tag at random (the first 2 caught in the
108 corral), except in the first vaccinated group (YT) where 8 sheep were chosen. In
109 the control region without vaccination the selection of animals for blood
110 sampling was similar, involving the selection of 3 cohorts of sheep of similar
111 ages to those in the vaccinated groups (lamb, 3-4 years old, old sheep). Sera
112 were also obtained from an additional group of control lambs which were
113 derived from an area known to be not endemic for echinococcosis (Puerto
114 Madryn, Argentine) (total 4 control groups). Ten ml of blood sample from jugular
115 puncture were obtained (Table 12). Blood samples were centrifuged to obtain
116 serum and were maintained at 5° C before they were sent to the laboratory
117 where the samples were kept at -20°C.

118 EG95 specific antibody responses were evaluated in serum samples
119 from 341 animals. Of these, 178 belonged to the vaccinated group and 163
120 corresponded to the non-vaccinated control groups (Table 1).

Anti-EG95 responses were determined using similar procedures to those described by Heath and Koolaard [11] and Poggio et al. [12]. In summary, the EG95 antigen for ELISA was prepared by expressing EG95-6HIS in *E. coli*, and purifying the construct with Protino Ni-TED/IDA (Macherey-Nagel, Düren, Germany). Optimal antigen and conjugate concentrations were determined using chequer-board titrations of positive and negative sera. A 50µl volume of EG95-6HIS at 1µg/ml in coating buffer was added to each test well of Nunc Immunosorb ELISA plates and incubated at room temperature overnight. Plates were washed and blocked with 300 µl/well of blocking solution (900 mL Phosphate-Buffered Saline, 100 mL adult horse serum, 1% phenol red) for 1 hour at room temperature. Sera were assayed in blocking solution at a dilution of 1:200. Plates were washed 3 times and 100µl of donkey anti-sheep IgG conjugated to horse radish peroxidase (Invitrogen, Carlsbad, CA. USA.), 1:3000 in blocking solution was added and incubated at room temperature for 1hr. After washing the plates, 100µL of ABTS substrate (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) 0.5mg/ml in 70mM citrate phosphate buffer, pH 4.2) was added to each well. Plates were incubated in the dark for 20 minutes, stopped by the addition of 50µl of 2% sodium fluoride and the plates subsequently read at 405nm using an automated ELISA plate reader.

Statistical analyses: median and interquartile range value of the optical density (OD) at 405nm plus standard deviation was estimated to specific EG95 IgG level in serum samples from vaccinated and non-vaccinated groups.

Unidirectional ANOVA test (variance analysis) were used for comparing the ELISA OD values among 6 different cohorts of vaccinated and 4 non-vaccinated sheep, as well as Fisher and Tukey test. Student t-test were used

for comparing absorbance values among all vaccinated and non-vaccinated sheep. All tests were performed using MINITAB 16.

Regarding the ethical concerns around the treatment protocol of sheep used in this study, the experimental protocols were approved by the Research Committee at the National University of La Pampa, School of Veterinary Medicine. The study was conducted adhering to the regulations of the National Animal Health Service concerning animal welfare.

3. Results

Median ELISA EG95 absorbance values and interquartile range from of vaccinated (0.828, 0.530) and non-vaccinated (0.218, 0.138) groups of sheep are shown in Figure 1. Student t-test analyses indicate significant differences between OD_{405nm} values from EG95 immunized and control groups of sheep ($p< 0.0001$). Significant differences are also evidenced in ANOVA analyses which indicate significant differences in the OD_{405nm} values when 4 cohorts of non-vaccinated animals and 6 cohorts of vaccinated sheep ($F= 75.5$, $p<0.001$, R^2 67.3) were compared. ANOVA also demonstrated significant differences in the OD_{405nm} values when only the 6 cohorts of vaccinated sheep ($F=17.8$, $p<0.001$) were considered.

Post hoc ANOVA analysis revealed no differences among non-vaccinated animals of different ages, however differences between vaccinated and non-vaccinated control groups were evident as well as some variations among vaccinated animal groups over time.

Specific IgG responses to EG95 vaccination are shown in Figure 1 as the median OD405nm values with interquartile range in EG95 vaccinated groups

(n=178) and age-matched non-vaccinated control sheep (n=163). An anti-EG95 antibody response was induced in all groups of immunized sheep, 4 times higher (0.828) than the median observed in the control groups (0.218). More dispersion was detected between the most extreme values within the dataset of vaccinated sheep compared with non-vaccinated control groups which show lower variability (Figure 1). EG95 antibody response~~s~~ in sheep from different cohorts of non-immunized and vaccinated groups are shown in Figure 2 and reveal the sustained increase in response seen in animals following the third immunization.

4. Discussion

Data described here indicate that following a third vaccination with the EG95 vaccine at 1 year of age the specific IgG responses detected in the serum of sheep increased to a level greater than that seen following the second immunization and that this response was maintained longitudinally over time, for at least 5 years. Protection against infection afforded by the vaccination has been detailed by Larrieu et al. [14]. Four sheep in the yellow tag (YT) group were found to harbour a total of 6 hydatid cysts, all of which were small (1 x 1.3 cm to 0.2 x 0.2 cm). Of these, 2 were found in the liver (one fertile), and 4 in the lung (average 0.3 cysts per animal). In 13 non-vaccinated animals, 47 hydatid cysts were detected (1.4 cysts per animal), some larger than 5 cm. A statistically significant difference was demonstrated in the number of cysts found in sheep from the control group and the vaccinated group (p=0.02) [14]. These data support previous results which suggest that the EG95 vaccine is suitable as an effective control measure to reduce the level of transmission of *E.*

196 *granulosus* in sheep and likely to be reflected in a decreased incidence of
197 human infection [8,13,14].

198 In a review publication, Heath et al. [8] refer to a safety and efficacy trial
199 of the EG95 vaccine involving 50,000 and 100,000 lambs in Qinghai and
200 Xinjiang Provinces of China. Two injections were described as inducing a high
201 level of antibody that protected the animals (85%) against a natural challenge.
202 The vaccine remained effective for at least 12 months and specific serum
203 antibodies remained detectable. Following a third injection given 6-12 months
204 after the second vaccination, a higher level of antibody was induced and up to
205 100% of protection was reached. These data are consistent with our findings
206 here that specific serological responses can be detected to the vaccine for at
207 least a year following two immunizations in lambs and that the responses are
208 boosted, and long lasting, following a third immunization of animals at
209 approximately 1 year of age.

210 Heath and Koolaard [11] presented evidence supporting a direct
211 association between the titre of anti-EG95 antibodies and the level of protection
212 against an experimental infection with *E. granulosus*. In the antibody assay
213 used by Heath and Koolaard [11], a specific anti-EG95 level recorded at 1:400
214 dilution of OD_{405nm} 1.0 indicated a protective level. Poggio et al. [12] identified a
215 similar level of protection associated with animals having an OD_{405nm} ≥1.0 and a
216 reduced level of protection (average 84%) in animals that had lower levels of
217 antibody (around OD_{405nm} around to 0.7). Here we have identified specific
218 antibody responses in sheep after 3 doses of EG95 vaccine in field conditions
219 and in a control program which correspond with levels shown previously to be
220 protective against experimental challenge infections [12].

A greater variability in the OD values was observed between the dataset of vaccinated sheep, compared with non-vaccinated control groups which show lower variability. The variability in the vaccinated animals reflected a wide variation in responses induced by the vaccine in different animals. One of the contributing factors was that not all animals received the three scheduled doses of vaccine. The native communities where this control program was undertaken are remote and have rudimentary infrastructure. In many instances, it was also not possible for the farmers to have all their animals available for vaccinations when they were due. For this reason, some animals missed one or more of their scheduled vaccinations. As well as being reflected in variability in the serological responses among vaccinated animals, unreliability in being able to deliver immunizations to individual animals is likely to have contributed to the lower level of protection observed in the field trial compared to the results following experimental challenge infection in vaccinated sheep [12]. This trial was undertaken using procedures that would be expected to apply if EG95 vaccination were implemented as a routine procedure for the on-going prevention of *E. granulosus* transmission. In such circumstances, animals would not be expected to be identifiable individually, and some animals may fail to be mustered and miss one or more of their scheduled animal health treatments, eg vaccinations.

In the field trial described here, a reduction in specific anti-EG95 antibodies was seen in the 3rd and 4th years of the study (Figure 2) coinciding with the period following eruption of the Puyehue volcano in Chile that produced animal malnutrition and mortality.

More information is required to determine whether there may be a need for a further booster vaccination in animals during their fourth or fifth years to provide protection to those animals that survive beyond this age. The addition of a V4 booster, may be useful in (a) protecting animals that may miss one of their previous vaccinations and (b) in possibly further lowering the biomass of potentially-infective cysts available for dogs.

5. References

[1] E. Larrieu, F. Zanini, F.

Critical analysis of cystic echinococcosis control programs and praziquantel use in South America, 1974-2010.

Pan Am. J. Public Health. 31 (2012), pp. 81-87.

[2] C. F. Pavletic, E. Larrieu, E. Guarnera, N. Casas, P. Irabedra *et al.*

Cystic echinococcosis in South America: a call for action

Pan Am. J. Public Health, (2016) in press.

[3] C.M.Budke, P. Deplazes, P.R. Torgerson.

Global socioeconomicimpact of cystic echinococcosis.

Emerg. Infect. Dis. 12 (2008), pp. 296-303

[4] E. Larrieu, M.T. Costa, G. Cantoni, J.L. Labanchi, R. Bigatti, R. *et al*

Control program of hydatid disease in the province of Rio Negro Argentina. 1980-1997.

Bol. Chil. Parasitol. 55 (2000), pp. 49-53

[5] J.C. Salviti, M. Sobrino M. Del Carpio, C. Mercapide C, L. Uchiumi, *et al.* **Hydatidosis: Ultrasonographyc screening in the Río Negro Province 25 years after the first screening**.
Acta Gastroenterol. Latinoam. 44 (2015) pp. 311-315

[6] E. Larrieu, M. Del Carpio, C, Mercapide, J.C, Salvitti, J. Sustercic*, et al.* **Programme for ultrasound diagnoses and treatment with albendazole of cystic echinococcosis in asymptomatic carriers: 10 years of follow-up of cases**. Acta Trop. 117 (2011) pp. 1-5.

[7] C. Gauci, D. Jenkins, M.W. Lightowlers, **Strategies for optimal expression of vaccine antigens from taeniid cestode parasites in Escherichia coli**.
Mol. Biotechnol. 48 (2011) pp. 277-289.

[8] D.D. Heath, O. Jensen, M.W. Lightowlers, **Progress in control of hydatidosis using vaccination-a review of formulation and delivery of the vaccine and recommendations for practical use in control programmes.**
Acta Trop. 85 (2003) pp. 133-143.

[9] M.W. Lightowlers, O. Jensen, E. Fernandez, J. Iriarte, J.D. Woollard, *et al.* **Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccine in sheep**.
Int. J. Parasitol. 29 (1999) pp. 531-534.

[10] M.W. Lightowlers **Cestode vaccines: origins, current status and future prospects**.
Parasitology 133 (2006) pp. 27-42.

[11] D.D. Heath, J. Koolaard,

Serological monitoring of protection of sheep against Echinococcus granulosus induced by the EG95 vaccine.

Parasite Immunol. 34(2012 PP. 40-4

[12] T.V. Poggio, O. Jensen, M. Mossello, j. Iriarte, H.G. Avila*, et al*.

Parasite Immunol. doi: 10.1111/pim.12325. (2016) [Epub ahead of print]

[13] E. Larrieu, E. Herrero, G. Mujica, J.L. Labanchi, D. Araya, *et al.*

Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: Early impact and preliminary data.

Acta Trop. 127 (2013) pp. 143:151

[14] E. Larrieu, G. Mujica, G. C.G. Gaucci, K. Vizcaychipi, T.V. Poggio, *et al.*

Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: second study of impact.

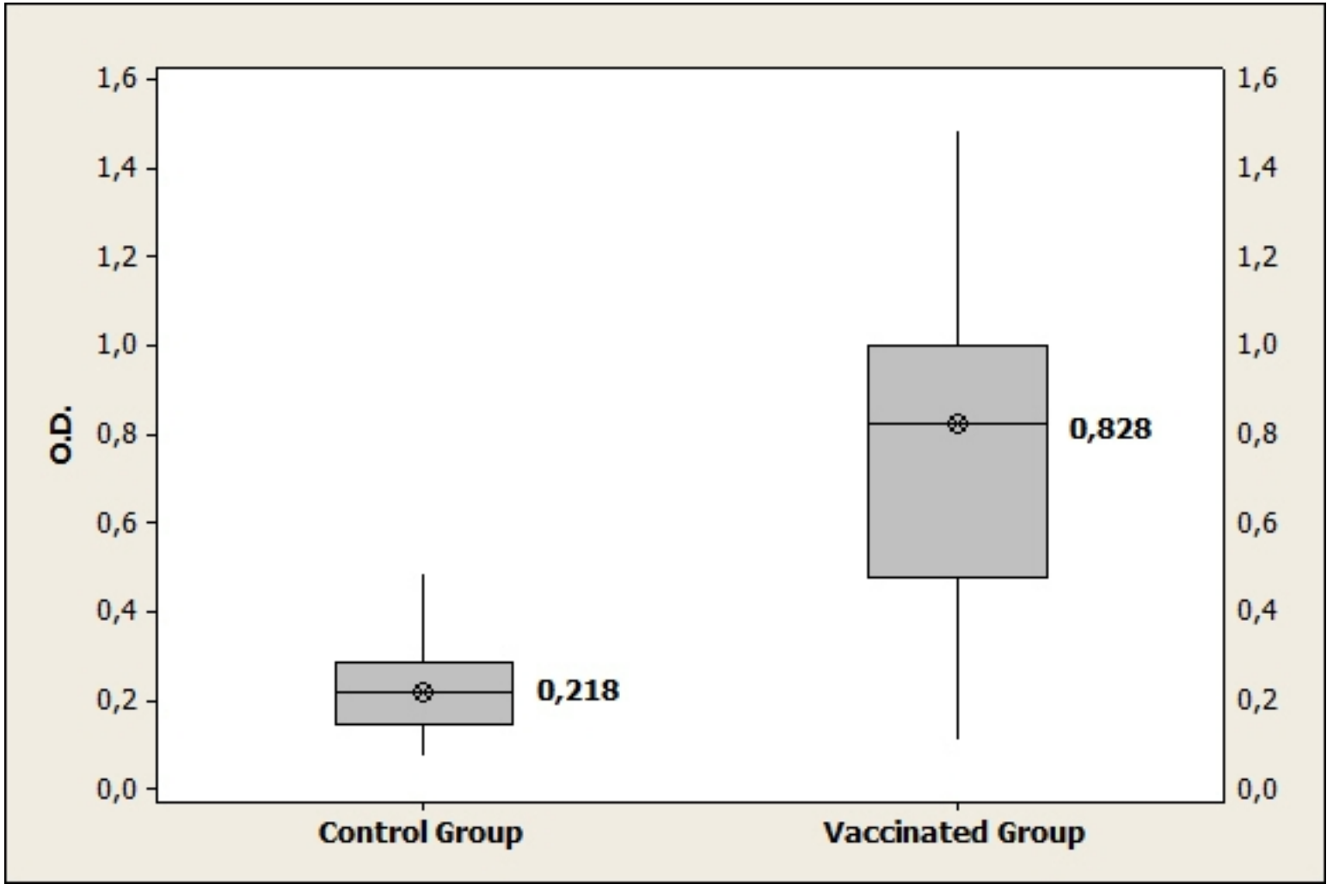
PLOS Neg. Trop. Dis. e0004134 (2015) pp. 1:10

Conflict of interest statement

All authors have not any actual or potential conflict of interest

Figure 1. Median, interquartile range and range of anti-EG95 specific IgG serum antibody responses (OD) measured in EG95 vaccinated and non-vaccinated sheep in 2015, in Negro Province, Argentina.

Figure 2. Median, interquartile range and range of anti-EG95 specific IgG serum antibody responses (OD) measured in different cohorts of EG95 vaccinated and non-vaccinated sheep in 2015 in Negro Province, Argentina.


O.D.
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,218
0,828
Control Group
Vaccinated Group

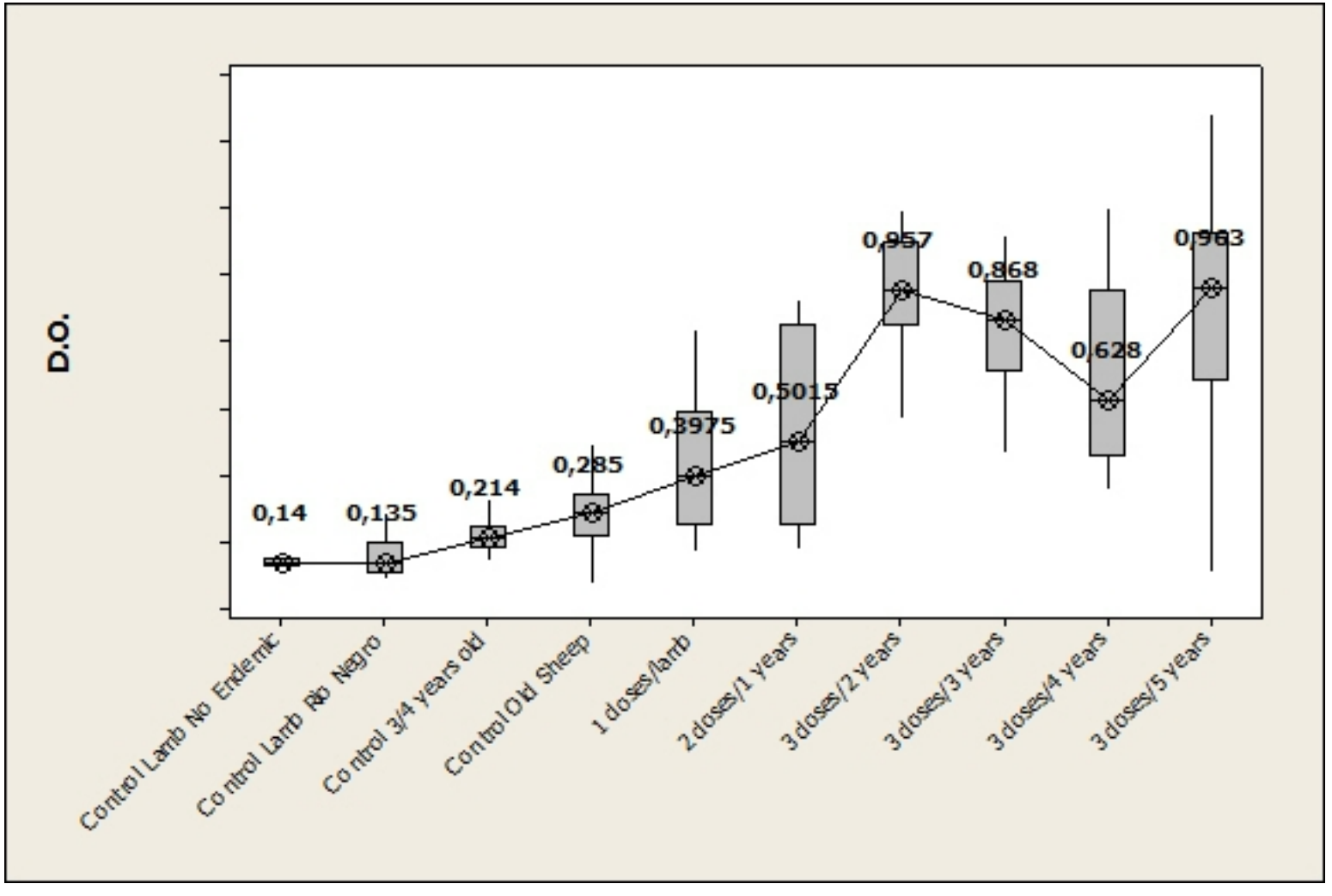

D.O.
0,14
0,135
0,214
0,285
0,3975
0,5015
0,957
0,868
0,628
0,963
Control Lamb No Endemic
Control Lamb Rio Negro
Control 3/4 years old
Control Old Sheep
1 doses/lamb
2 doses/1 years
3 doses/2 years
3 doses/3 years
3 doses/4 years
3 doses/5 years

Table 1. Characteristics of non-vaccinated and EG95 vaccinated sheep between 2009 and 2015, in the Rio Negro Province, Argentina in 2015

Sheep Cohorts (tag code**)	# Blood samples in 2015	Age in 2015	Vaccination schedule in 2015	Observation	Action
Vaccinated (YT)	89	5/6 years old	3	December 2009 first doses lamb YT*	2009 Initial diagnoses (Larrieu et al, 2013)
Vaccinated (RT)	13	4 years old	3	December 2010 first doses lamb RT*	
Vaccinated (BUT)	15	3 years old	3	December 2011 first doses lamb BUT*	
Vaccinated (GT)	14	2 years old	3	December 2012 first doses lamb GT*	2012 First impact study (Larrieu et al, 2013)
Vaccinated (WT)	12	1 years old	2	December 2013 first doses lamb WT*	
Vaccinated (BKT)	35	60 days	1	December 2014 first doses lamb BKT8*	
Total vaccinate***	178				2015 Second impact study (Larrieu et al, 2015) and evaluation EG95 specific antibody responses in all vaccinated group
Control old sheep	81	5/6 years old	0	Work area	
Control sheep	25	3/4 years old	0	Work area	
Control lamb	47	30/60 days	0	Work area	
Control lamb NE	10	30 days	0	Non endemic area	
Total unvaccinated****	163				2015 evaluation EG95 specific antibody responses in all no vaccinated group
TOTAL	341				

*Age at vaccination: 30, 60 and 365 days in first, second and third doses respectively

**Tag Code: BKT (Black), WT (White), (GT) Green, BUE (Blue), RT (Red), YT (Yellow)

*** sheep from Rio Chico Abajo, Anecon Grande, Mamel Choique **** sheep from Blancura Centro and Puerto Madryn

Pilot field trial of the EG95 vaccine against ovine cystic echinococcosis in Rio Negro, Argentina: humoral response to the vaccine

Vaccination of potential intermediate hosts of *E. granulosus* with the EG95 vaccine could be used to reduce *E. granulosus* transmission

Responses after the third vaccination at 1-1.5 year of age induce an increase in titre greater than seen following the second immunization

Unvaccinated controls also show an increase in OD with age, although, even in animals of six years old, OD values are lower than those of lambs with a single dose of vaccine

Antibody OD levels observed in sheep with 3 doses of EG95 showed a serological pattern (OD 0.94) required for a higher protection

In the future it will be important to demonstrate the effect of sheep vaccination on transmission to dogs