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

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Highlights

A field trial of the EG95 vaccine against cystic echinococcosis was initiated in Rio Negro, Arg.

The prevalence of ovine echinococcosis is 61.3% in old animals at necropsy.

Preliminary assessment identified a reduction in infection in the vaccinated animals

Abstract

Cystic echinococcosis is endemic in the Rio Negro province of Argentina. After 30 years of control using praziquantel in dogs the transmission rate to humans and sheep has decreased significantly, however transmission persists. The objective of the study is to assess the impact of the inclusion of the EG95 vaccine for sheep in the control program, including analysis of the vaccine's operative feasibility in field conditions. The vaccine was applied in an area comprising four communities of native people including 79 farms with 3146 lambs and 311 dogs in total. Seventy one farms were designated as control areas where no vaccinations were undertaken while vaccinations of lambs undertaken on 91 farms. Lambs received two vaccinations with the EG95 vaccine followed by a single booster injection when the animals were 1-1.5 years of age. Farm locations were defined using GPS coordinates for the houses. Evidence for *Echinococcus granulosus* transmission was monitored by coproantigen ELISA on samples of dog faeces, by *E. granulosus*-specific PCR using soil samples, and anti-*E. granulosus* antibody assessments in sera from 2-4 teeth lambs, purgation of dogs to detect *E. granulosus* worms and necropsy on adult sheep. Before the vaccine was introduced, 26.2% of sheep with 2-4 teeth were positive using ELISA/WB, the prevalence decreased to 7.8% at the third year following use of the vaccine. Necropsy of animals older than 6 years (not vaccinated) showed that 66.1% of animals were infected with *E. granulosus*. In dogs, 4% was found positive for *E. granulosus* using arecoline purgation and 24.7% of the farms were infected using coproELISA/WB. During the first year of vaccination 2721 lambs received the first vaccine dose and 2448 received a booster. In the second year 2138 lambs were initially vaccinated and 1745 received a booster, and 1308 animals received the third dose. During the third year 1110 lambs received the first dose from which 539 received a booster and 723 animals received the third dose. An analysis of advantages and limitations of the diagnostic techniques used and the ability of the geospatial analysis to detect risk area are included. Based in the immunodiagnostic techniques, the EG95 vaccine has been able to prevent the infection in animals up to 3 years old. Also, the difficulties in the field for the correct vaccine administration and the social features and habits that may impact on echinococcosis control are included in the analysis.

1. Introduction

Cystic echinococcosis (CE) is a parasitic zoonosis caused by infection with a taeniid cestode parasite, *Echinococcus granulosus*. The parasite requires two mammalian hosts to complete its life cycle. In South America the most important definitive host is the dog (which develops the adult tapeworm stage) and the most common intermediate host is the sheep (which develops the larval stage). Goats, pigs and cattle can be involved also as intermediate hosts (Eckert et al., 2001; Larrieu and Zanini, 2012). CE is one of the most prevalent zoonoses in Argentina, causing a significant burden for the health system due to the high costs of surgery and days spent in hospitals. It also causes losses in livestock due to the condemnation of infected viscera and decreases in production of wool, milk and meat (Budke et al., 2006).

Since the introduction of the cestocidal drug praziquantel, control programs for CE have been based largely on dog treatment with this drug every 45 to 90 days in an attempt to eliminate the adult tapeworms. Hypothetically, if all dogs in a region were treated every 45 days the risk of transmission to humans and animals as intermediate hosts would decrease rapidly to reach a level where transmission was totally interrupted. In situations such as in the Rio Negro Province of Argentina, where sheep are responsible for acting as intermediate hosts, renewal of the sheep with animals born after interruption of transmission would cause the elimination of *E. granulosus* (Eckert et al., 2001).

However, where CE control has relied on frequent treatment of dogs with praziquantel, more than 10 years of intensive, compulsory intervention has been required before CE transmission reached a low level and almost 30 years has been required to achieve freedom from the disease (Craig and Larrieu, 2006). In other areas where CE control activities have been undertaken in the past century, the infrastructure required to treat the dogs 8 to 12 times per year for a long period of time (10 years or more) has not been sustainable, often because CE endemic areas are the poorest in each endemic country (Larrieu and Zanini, 2012).

A control program for CE started in 1980 in the Rio Negro Province based on treatment of dogs with praziquantel every 90 days, 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. Education activities were also undertaken in the community concerning the nature of the disease, lifecycle and the need to prevent dogs gaining access to sheep offal. Serological testing and untrasonography were undertaken on the human population in order to effect early diagnosis of CE infections (Larrieu et al., 2000; Larrieu et al., 2011; Larrieu and Zanini, 2012). The program has been successful in decreasing the prevalence of *E. granulosus* in dogs and humans, although a proportion of infected sheep remain and this has been sufficient to lead to a continued incidence of CE in children (Larrieu et al., 2001; Larrieu et al., 2000).

Vaccination of potential intermediate hosts of *E. granulosus* with the EG95 recombinant vaccine (Heath et al., 2003; Lightowlers et al., 1996) could potentially be used to reduce the level of *E. granulosus* transmission and decrease the incidence of human infections (Bethony et al., 2011). Torgerson (2003, 2006) and Torgerson and Heath (2003) have used mathematical models to predict the impact of various options for control of CE and considered that a program involving vaccination of intermediate hosts together with 6-monthly treatment of dogs with praziquantel would decrease the time needed to achieve control of disease transmission.

The EG95 vaccine is a recombinant protein cloned from mRNA from the oncosphere life cycle stage of the parasite. The vaccine comprises the EG95 protein together with the adjuvant Quil A. Two immunizations in young animals protects against a challenge infection with *E. granulosus* by inducing specific antibodies against the oncosphere, eliminating it before it can establish and develop in the tissues of the intermediate host. EG95 is a defined protein, non infectious, non toxic and is produced using genetic engineering and expressed in *Escherichia coli* (Heath et al., 2003; Lightowlers et al., 1999; Lightowlers et al., 1996). However, other than some data about the use of the vaccine in China (Heath et al., 2006; Heath et al., 2003), there is little published information about the impact of the EG95 vaccine when used in field conditions nor about potential problems that could arise when the vaccine was applied on a large scale in livestock flocks (Larrieu and Zanini, 2012).

In order to assess the impact of the introduction of the EG95 vaccine in the control program in Rio Negro, the authorities in charge of the program decided to trial the introduction of the vaccine as an additional control tool. It is considered that optimal protection with the EG95 vaccine would be afforded by two immunizations in lambs

followed by annual booster immunizations (Heath et al., 2003). However the resources available for hydatid control in Rio Negro were insufficient to support the requirements of this regime and it was decided to undertake a vaccination program in which lambs received two vaccinations followed by a single booster immunization when the animals were 1-1.5 years of age. The objective of this work was as assessment of the baseline data in the regions selected for the control programme with vaccination and a preliminary assessment of the effects of EG95 vaccination on lambs born following the introduction of the vaccine. Secondary objectives include the assessment of the advantages and disadvantages of the diagnostic tests available for epidemiological surveillance of CE and also an analysis of the usefulness of the geospatial analysis in the CE control activities.

2. Materials and Methods

2.1 Work Area

The regions chosen for the program were Anecon Grande, Rio Chico Abajo, Nahuel Pan, Manuel Choique, Blancura Centro and Lipetren. Each farm was defined as an Epidemiologic Unit (EU), each of them contained a house. The geographic region was the Rio Negro Province in Argentina comprising in total an area of 5820 Km^2. The geographic position of every EU was localized to create a geographic information system (GIS) (figure 1).

In these communities there are five health centres, with each of them employing a sanitary agent responsible for the first contact of the centre with the families. They are located in Manuel Cheque (latitude -41.7777 longitude -70.1369), Anecon Grande (-41.3215 -70.2742), Rio Chico abajo (-41.7098 -70.4761), Nahuel Pan (-41.9004 -71.4932) and Mencue (-40.4238 -69.6146).

2.2 Initial diagnostics before introduction of EG95 in the control programme:

Two meetings were held with the communities and their higher authority (Lonco) to inform the activities involved in the control program and and to provide information about the safety of the vaccine for animals and people. Each EU was visited to explain the activities and to collect information about the number of animals in each area.

A baseline for infection in the intermediate and definitive hosts and also in the environment was established. The information was used to assess the impact of the introduction of the vaccine in the control program.

Methods used were: coproELISA used as screening and confirmed with Western Blot in dog faeces (Cavagion et al., 2005; Guarnera et al., 2000). ELISA as screening and confirmation with Western Blot in sheep sera (Gatti et al., 2007); PCR was used for soil samples from each EU (Cabrera et al., 2002) for the detection of *E. granulosus* contamination. Direct methods used were necropsy of sheep with histological confirmation (Cabrera et al., 2003) and arecoline purgation in dogs (Perez et al., 2006).

EU selected for the baseline survey were selected at random. The size of the sample (''n'': 109 farms) was determined with the following parameters: 95% confidence level and 20% error margin. The arecoline purgation test was applied in dogs brought by the owners (not randomized).

CoproELISA/WB: fresh faeces were collected directly from the ground from each EU. One sample was collected for every 2 dogs. They were collected in plastic containers without fixatives and sent to the Laboratory for Environmental Health (San Carlos de Bariloche) to be tested by copro/ELISA. The samples that tested positive were sent for confirmation by WB to the National Institute of Microbiology (ANLIS/MALBRAN) following procedures previously described (Guarnera et al., 2000). The presence of one positive sample classified the EU as infected.

With this variable ("dog faeces coproELISA/WB positive-negative") cluster and atypical value (Anselin and Morans I) was estimated. The buffer tool was applied for determining spatial relation between negative EU that are in the influence's zone of a positive EU at within a distance of 2 kilometres (maximum area exposed to infected dogs)

ELISA/WB: 10 ml of blood from jugular puncture were obtained from lambs with 2-4 teeth. Samples were centrifuged to obtain sera and kept at 5°C before they were sent to the Laboratory of Zoonoses, Viedma, Rio Negro where the samples were kept at -20°C. ELISA was performed as previously described (Gatti et al., 2007).

PCR: soil samples (300g per house) were taken in each EU from areas near where the dogs sheltered. Soil was deposited in plastic bags and processed at the National Institute of Microbiology (ANLIS-MALBRAN) following procedures previously described (Cabrera et al., 2002).

Necropsy: animals > 6 years old were purchased from farmers and euthanized at the local slaughterhouse in Ingeniero Jacobacci; the slaughterhouse was used exclusively for trial purposes on the days that these animals were analysed. The presence of at least one viable CE cyst classified the animal as positive. Calcified and contaminated cysts were collected for histological confirmation.

Dogs concentration by arecoline purgation was carried out in each of the 5 works area and the purge examined for the presence of *E. granulosus* using a tray with a dark surface (Perez et al., 2006).

2.3 Vaccination and other CE control activities

Four teams were charged with vaccinating the sheep, each of them comprised 2 veterinarians from the Minister of Health and one sanitary agent from each community. The EG95 vaccine used was produced by the University of Melbourne (Gauci et al., 2011); 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 was injected subcutaneously in the neck of the animal. Vaccine containers with vaccine already diluted were kept in coolers while travelling between the different EUs.

Two different groups were assigned to different treatment types. One group comprising 71 EU of Blancura Centro and Lipetren regions, was established as control EU where no vaccinations were undertaken. In the treatment group, comprising 79 EU of Anecón Grande, Mamuel Choique, Nahuel Pan and Rio Chico abajo regions, lambs received two initial immunizations with EG95 (the first dose was applied to animals at 30 days of age and the second dose at 60 days of age before weaning) and also received a booster immunization at approximately 1-1.5 years of age. Lambs born on these EU in subsequent years would undergo the same vaccination treatments until the end of the trial.

In one EU area in which vaccination was employed a different treatment type was required. In Nahuel Pan, 13 EU, rams are run permanently with the ewes such that there is not a clear breeding season when lambs are born. In this region the ewes were vaccinated with one dose of EG95 during the period December-January and also the lambs were vaccinated with the standard treatment. The objective of this treatment was to generate colostral immunity in lambs born after vaccination.

In order to facilitate the vaccinations, animals were herded into paddocks by the farmers following a visit and instructions from one of the participating community sanitary agents and an announcement was made to the community of the impending visit by the project staff via "National Radio". All the vaccinated animals were ear tagged using a different colour tag for each year of the project. Goats were not included in the control programme with vaccination and farmers that exclusively kept goats were not included in the project, however the dogs associated with these properties maintained the 4x/year dog dosing schedule. Vaccinations were carried out by 4 teams, one of which visited each property twice each year, approximately one month apart, for the purpose of giving the two immunizations to new lambs and to re-vaccinate lambs born and vaccinated the previous year. For the duration of the trial, other CE control activities (praziquantel treatment of dogs every 3 months) continued throughout the control trial and surrounding areas.

2.4 Study of impact after vaccination

A preliminary study was carried out on sera from 2-4 teeth lambs during the third year following the initiation of the vaccination program using ELISA/WB serology. Results were compared with sera from animals of the same age from the control area.

2.5 Statistic and spatial analysis

Statistical comparisons between groups control and vaccination were performed using the Chi Square Test and statistical comparisons between different techniques were evaluated using the Fisher Exact Test (p value 0.05) and confidence intervals of 95% (CI95%) were estimating using EPIDAT 3.1 (Xunta of Galicia, Spain)

ArcGis 10.0 with the Spatial Analyst extension (ESRI) and SatScan 7.01 were used for geospatial analysis..

3. Results

3.1. Initial diagnostic in areas control and vaccination:

An initial census (2009) of the trial identified 150 sheep farmers in the area used for vaccination and control. Animals in these areas were 16511 sheep, 4696 lambs and 452 dogs (Table 1). The EU in which vaccination was performed included 79 farmers

which incorporated 3146 lambs and 311 dogs. Data concerning evidence for *E. granulosus* transmission in each of the regions chosen for inclusion in the vaccination program are summarized in Table 2. Statistical comparisons of the prevalences indicated in Table 2 for each of the different techniques were all not significant ($p > 0.05$) when comparing the results from the Control Area and the vaccination A, with the exception of results obtained from PCR on soil samples ($p < 0.05$).

In relation with the different techniques, the prevalence of CE in 2-4 teeth lambs was 25.7% determined using ELISA/WB whereas 61.3% of the animals older than 6 years were positive using the same test and 66.1% were positive at necropsy. No significant differences were found between the prevalence data obtained for aged sheep using the two methodologies ($p>0.05$).

The prevalence of *E. granulosus* infection determined in dogs using arecoline purgation was 4 %. Using Copro ELISA/WB on dog faeces, 11.2% of the samples were positive. Differences found between both tests were statistically significant ($p<0.05$).

Spatial analysis in Rio Chico abajo – Mamuel Choique showed a cluster of 2 EU infected with values $z >1.96$, and p values <0.05 (Figure 2). Application of buffer analysis allowed the definition of geographic areas with negative results that might suffer the influence of positive neighbour areas (Figure 3).

PCR in soil showed that 13.5% of the EU was infected and 24.7% of EU had 1 dog faeces positive. Difference in the results using copro/WB and PCR were no significant ($p=0.05$, but only 2 EU positives in copro/WB were also positives in PCR.

The average n of carcases was mean 13.6 +/- SD 2.2 kg. The infected animals were heavier (13.8+/-2.3) than the no infected animals (13.2+/-2.3), however the difference were not statistically significant ($p>0.05$).

3.3. Vaccination

Vaccination began in December 2009. The numbers of animals involved in the vaccinations during the 3 years in which the program has been continuing are shown in Table 3. In the first year 2721 lambs received an initial dose of vaccine and 2448 received the second immunization approximately one month later, representing 86.5/77.8% of the animals respectively and 93.7/94.9% of the farmers. In the second year 2138 new lambs received the first injection and 1745 received the second immunization, representing 67.4/55.0% and 78.3/73.5% of coverage respectively. Of the animals receiving vaccination in the first year, 1308 received a booster

immunization when the animals were approximately 1-1.5 years of age, 48.1% of the animals initially vaccinated. In the third year 1110 new lambs received the first vaccine dose and 539 of them received the second dose (coverage 94.1/45.7% of the animals and 94.0/58.2% of the farmers) out of a total number of 1136 new lambs. The third dose was applied to 723 lambs, 22.8% in relation to the animals vaccinated with the first dose in 2010 (Table 3).

3.4. Study of impact

An initial assessment was made of the impact of the vaccine on infection levels in 275 two year old sheep based on ELISA/WB. Twelve of the 154 vaccinated animals were determined to be positive (7.8%) while in the control area 33 out of 84 sheep were found positive (39.3%). The differences between vaccinated and non vaccinated animals were significant ($p<0.05$) (Table 4)

4. Discussion

Six areas involved in the on-going CE control program in Rio Negro Province were selected for investigations into the impact of the EG95 vaccine. Comparison of various indicators of *E. granulosus* transmission in these areas obtained as base-line data prior to the initiation of vaccination activities in 2009 indicated that there was active transmission of the disease in all areas. Comparison of the areas selected for inclusion in the vaccination program and for inclusion as control, non-vaccination areas, found no significant differences with respect to all the measures of CE transmission that were assessed, excepted by PCR in soil (Table 2).

In the first year of the vaccination program 86.5% of the lambs born on the EU involved in the program received the first immunization and 77.8% of these received the second immunization, with approximately 94% of the EU participating in the program (Table 3). In the second year of the program a lower proportion of the animals that were scheduled to be vaccinated received the required immunizations; approximately half the lambs did not receive their second immunization and similarly approximately half the animals that received vaccinations in the first year did not received their booster immunization at 1 year of age. During the third year of the program the eruption of the Puyehue Volcano in Chile, after lying dormant for more than 50 years, affected the

programme. Ash falling over the work area led to the death of many animals and poor nutrition of many of the survivors. It also affected the access to the area.

At this time there are few data available that could predict the likely level of immunity to *E. granulosus* infection in sheep that had received two EG95 vaccinations as lambs and a single booster vaccination at 1-1.5 year of age, but no further immunizations. Similarly, it is difficult to predict the level of immunity that may be induced in animals that do not receive each of these scheduled vaccinations. Heath et al. (2003) suggest that some degree of immunity to infection is stimulated by a single dose of EG95 in lambs. The animals were tagged such that it will be possible to differentiate animals born each year of the vaccination control programme however they were not numbered tags and it is not known which individual animals received vaccination. Records are being maintained for each EU such that, where circumstances were such that one or more scheduled vaccinations did not take place for some reason (eg farmer unable to muster the animals), this information will be available for correlation with subsequent serology and necropsy data for these animals.

The control program for CE in Río Negro Province was developed by the Minister of Health in 1980 and was based mainly on dog treatment with anthelmintic drug. There were difficulties with implementation of the control program. Geographic isolation of the affected regions, adverse climatic conditions and the lack of vehicles adversely affected the program. These difficulties prevented some of the control areas being visited at some times of the year and this affected the coverage of dog treatments and other scheduled activities. However the control program proved to be successful since the prevalence in children (6 to 14 years old) decreased from 5.6% in 1986 (using ultrasonogrphy) to 0.3% in 2008 (Perez et al., 2006; Larrieu et al., 2011). But new cases were also diagnosed each year in children. In sheep the prevalence decreased from 61% at the start of the program to less than 20% (Larrieu et al., 2011), however it has remained around this percentage. Due to the persistence of the disease transmission, new control strategies were considered necessary, such as the vaccination of the animal intermediate hosts.

The diagnostic techniques used in this work to detect CE infection in sheep are generally not recommended (Eckert et al., 2001). However, some recent publications have found a good correlation between serology and necropsy examination for *E. granulosus* infection (Dueger et al., 2003; Gatti et al., 2007) and for this reason, serology has been used in the work reported here.

In relation to diagnosis of infection in dogs, the advantages and disadvantages of the arecoline purgation are well known. Advantages include it being 100% specific when worms are detected, and the test has value when the results can be demonstrated directly to dog owners so as to educate them about the parasite. However, the test has many disadvantages, including it being poorly sensitive, presenting some danger to the dogs and also being dangerous to the persons who have to handle infective purge material (Perez et al., 2006; Lahmar et al., 2007; Varcasia et al., 2011). In comparison, CoproELISA/WB is a simple, accurate and economic technique (Guarnera et al., 2000; Cavagion et al., 2005). Its disadvantages are that the samples must be transported to a laboratory and hence the results cannot be immediately demonstrated to a dog owner. In this work, CoproELISA/WB was used as a tool for determination of environmental contamination (EU with at least 1 dog infected) and was not used for diagnosis of infection in individual animals. The procedure used for sampling dogs allowed the chance of taking samples from the same dog (Guarnera et al., 2000).

PCR has been shown to be useful in the identification of *E. granulosus* from soil samples (Cabrera et al., 2002; Shaikenov et al., 2004; Mathis and Deplazes, 2006). In this work PCR was used to assess the environmental contamination in the same way as coproELISA/WB, however the results were different using both techniques analysing samples from the same EU. The reasons for these differences are unclear. The samples analysed by the two methods were not the same and were taken at different times, with the samples assessed in PCR being obtained a year before those analysed by coproELISA/WB. It is unknown how long a farm that is found to be positive in PCR, due to contamination with *E. granulosus,* remains positive after the level of contamination decreases as measured by a reduction in the incidence of infection in lambs.

An interesting finding was the similarity in weight of infected and non infected animals at necropsy. It has previously suggested that CE produces losses in meat production (Budke et al., 2006). However the revision of literature does not allow identification of significant experiments done to verify this theory.

The use of geographic information systems (GIS) have become more common as a tool for public health investigations due to the increase in number and quality of satellites used for terrestrial observation. These systems integrate the use of GPS information, spatial analysis and communications and provide an improvement in epidemiological analysis and prediction of future events, using remote sensors for

monitoring of a particular disease producing information that is important in designing a control program.

GIS have been not been extensively used for non vector borne disease, even less for parasitic disease like CE. For *E. multilocularis* in Germany, a study by Staubasch et al. (2001) related the geographic location of positive faecal fox samples with water sources, soil humidity and type of vegetation in images captured by a satellite. While in China, temporal and spatial analysis have been performed using images captured by satellite analysing the kind of plants found and the location of villages using GPS in studies for prevalence of human CE (Graham et al., 2004). Recently, Cringoli et al. (2007) built a model that analyses the spatial relation between cattle and buffalo farms infected and Brundu et al. (2012) used scan statistics with the Bernoulli model to detect and evaluate clusters of infected farms and also clusters of "non-cases".

The tools for cluster assignment (temporal association and tool for spatial infection) and buffer were defined based in the results from positive canine faeces in coproELISA/WB. They allowed the definition of risk areas due to density of infection, this facilitates the assignment of resources and control strategies focused not only to the EU positive but also to the surrounding areas.

The buffer tool was especially useful for establishing the impact of EU in the neighbour areas were dogs are not infected. This situation might affect the control program based in dog treatment and vaccination, for example in the case were positive EU are surrounding the control area, in this case specific studies must be performed to assess its influence in the transmission of the parasite.

There are factors that could hinder the effectiveness of vaccination that are not inherent to the vaccine itself, including the level of organization and funding for the control program, assuring an appropriate coverage of vaccination.

Other factors such as requirements for storage conditions for the reconstituted vaccine and the potential for *E. granulosus* transmission by a variety of different livestock host species, which, if incorporated in the vaccination program, would increase costs (Larrieu and Zanini, 2012). Also, the length of time between the parturition time and vaccination of lambs could favour lambs grazing contaminated pastures and being infected prior to vaccination. Nutritional status of vaccinated animals could have an influence on the response of the animal to the immunization. Vaccination of livestock requires farm infrastructure and must be completed in a short period of time so as to minimize costs. Although vaccination requires fewer interventions compared

with dog treatments per year (2 vaccinations versus 8 dog treatments) it also involves a higher number of animals compared with dog treatment (3146 lambs versus 311 dogs in this study).

Based on the immunodiagnostic techniques used in the present work, the EG95 vaccine has been able to prevent the infection in animals up to 3 years old. In the future it will be important to determine at necropsy if the vaccinated animals were protected by the vaccine during the animals' whole productive life. If this were the case, transmission to dogs would be prevented and therefore transmission of CE to humans would be eliminated.

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Figure 1.Geographic area for CE control program showing sheep farm in control and vaccination areas. Río Negro, 2010

Figure 2. Spatial analysis of the situation before vaccination with EG95: Detection of a positive cluster using positive coproELISA/WB in Rio Chico abajo – Mamuel Choique. Río Negro, 2009.

Figure 3. Spatial analysis: detection of buffer areas using positive coproELISA/WB in Rio Chico Abajo and Manuel Choique, Rio Negro, 2009.

Graphical abstract

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

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A field trial of the EG95 vaccine against cystic echinococcosis was initiated in the Rio Negro province of Argentina. Base-line data are presented for the field trial región as well as preliminary data on the effects of the vaccine's use on *Echinococcus granulosus* prevalence in sheep.

Table 1: Description of the regions in Rio Negro where the study was undertaken.

Area	Farms with sheep	Sheep (Number, range)	Lambs	Dog
Mamuel Choique	13	1306 15-228	452	41
Rio Chico abajo	39	5012 2-430	1656	173
Anecón Grande	14	2860 15-300	916	49
Nahuel Pan	13	205 7-57	122	48
Total: Vacunation area	79	9383	3146	311
Total: Control area	71	7128	1550	141
Total	150	16511	4696	452

Table 2. Diagnostic values for *Echinococcus granulosus* obtained as base-line data before the introduction of the EG95 vaccine in the Rio Negro control program during 2009.

Animal/technique		Control Area[a]			Vaccination area[a]			Total[b]				P *
Sheep/ ELISA/WB 2-4 tooth		168	44	26.2	84	22	26.2	256	66	25.7	(20.2 31.3)	1.0
Sheep/adults ELISA/WB		46	30	65.2	16	8	50.0	62	38	61.3**	(48.3 74.2)	0.2817
Sheep/adults Necropsy		46	32	69.6	16	9	56.3	62	41	66.1**	(53.4 78.7)	0.5711
Dogs/ Arecoline purgation		89	4	4.5	62	2	3.2	151	6	4.0***	(0.5 7.4)	0.6946
Environment/ - Copro ELISA/WB	IS	127	12	9.4	66	10	15.2	193	22	11.2***	(6.6 16.1)	0.2370
	EU	60	12	20.0	29	10	34.5	89	22	24.7****	(15.1 34.2)	0.1377
Environment/ - PCR soil		60	9	15.0	29	3	10.3	89	12	13.5****	(5.8 21.4)	0.0000

[a] Figures shown refer to the total number of samples tested, the number positive and the % positive, respectively. [b] Figures refer to the same as in [a] but with the addition of 95% confidence intervals in brackets. IS: Individual samples of canine faeces. EU: epidemiological unit (house). * Chi square test by statistical comparisons between groups control and vaccination. Fisher test by statistical comparisons between different techniques: ** P=035; *** P=0.009, **** P=0.05

Table 3. Number of animals vaccinated with EG95; Rio Negro, 2009-2012.

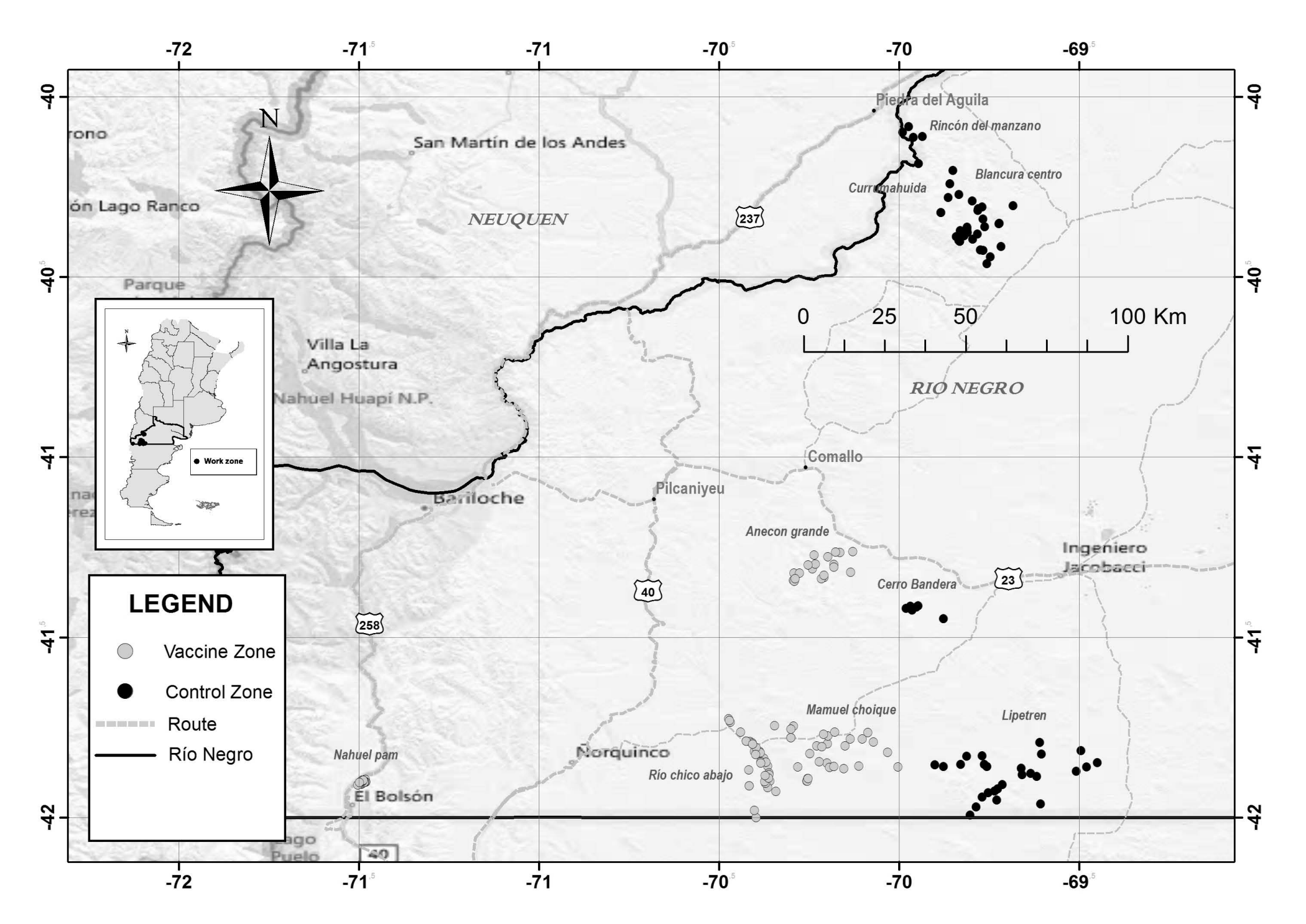
Vaccinations	2009-2010[a]			2010-2011[a]			2011-2012[a]		
	# Lambs	%V	%EU	# Lambs	%V	%EU	# Lambs	%V	%EU
Lambs 1st dose	2721	86.5	93.7	2138	67.4	78.3	1110	94.1	94.0
Lambs 2nd dose	2448	77.8	94.9	1745	55.0	73.5	539	45.7	58.2
Lambs 12 months[b]				1308	48.1*		723	22.8*	
Ewes	88			34					
Total sheep	5257			5225			2372		

[a] # Lambs: total number of animals that were vaccinated; %V: proportion of the animals available to be vaccinated that were actually vaccinated; %EU: the proportion of the epidemiological units (EU) that were scheduled to be include in the vaccination program that actually participated; [b] Booster immunization given to the previous year's lambs at approximately 12 months of age; *: based on first dose

Table 4. Diagnostic values for *Echinococcus granulosus* obtained with ELISA/WB after the introduction of the EG95 vaccine in the Rio Negro control program in 2012.

Sheep 2-4 tooth	Control Area[a]			Vaccination Area[a]			p*
Before EG959 vaccination	168	44	26.2	84	22	26.2	1.0
After EG95 vaccination	84	33	39.3	154[b]	12	7.8	< 0.0001
P**	0.05			0.001			

[a]: Figures shown refer to the total number of samples tested, the number positive and the % positive, respectively; * Chi square test by statistical comparisons between groups control and vaccination ; ** Chi square test by statistical comparisons between groups before and after vaccination ; [b]: > sample by < expected prevalence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Parque
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Nahuel Huapí N.P.
Bariloche
Comallo
Pilcaniyeu
Anecon grande
Ingeniero Jacobacci
Cerro Bandera
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Work zone
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Control Zone
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Lipetren
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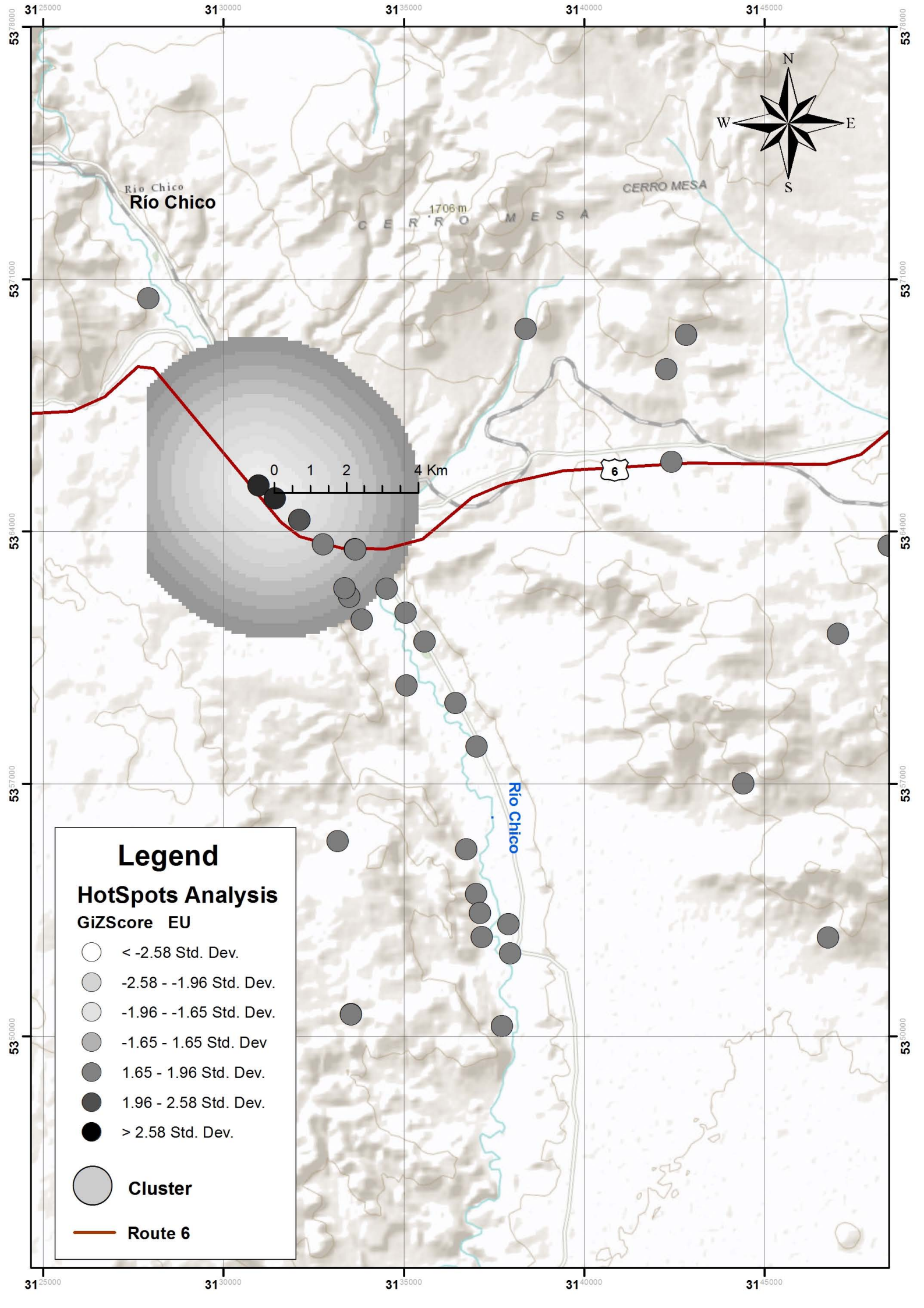
Río chico abajo
Nahuel pam
El Bolsón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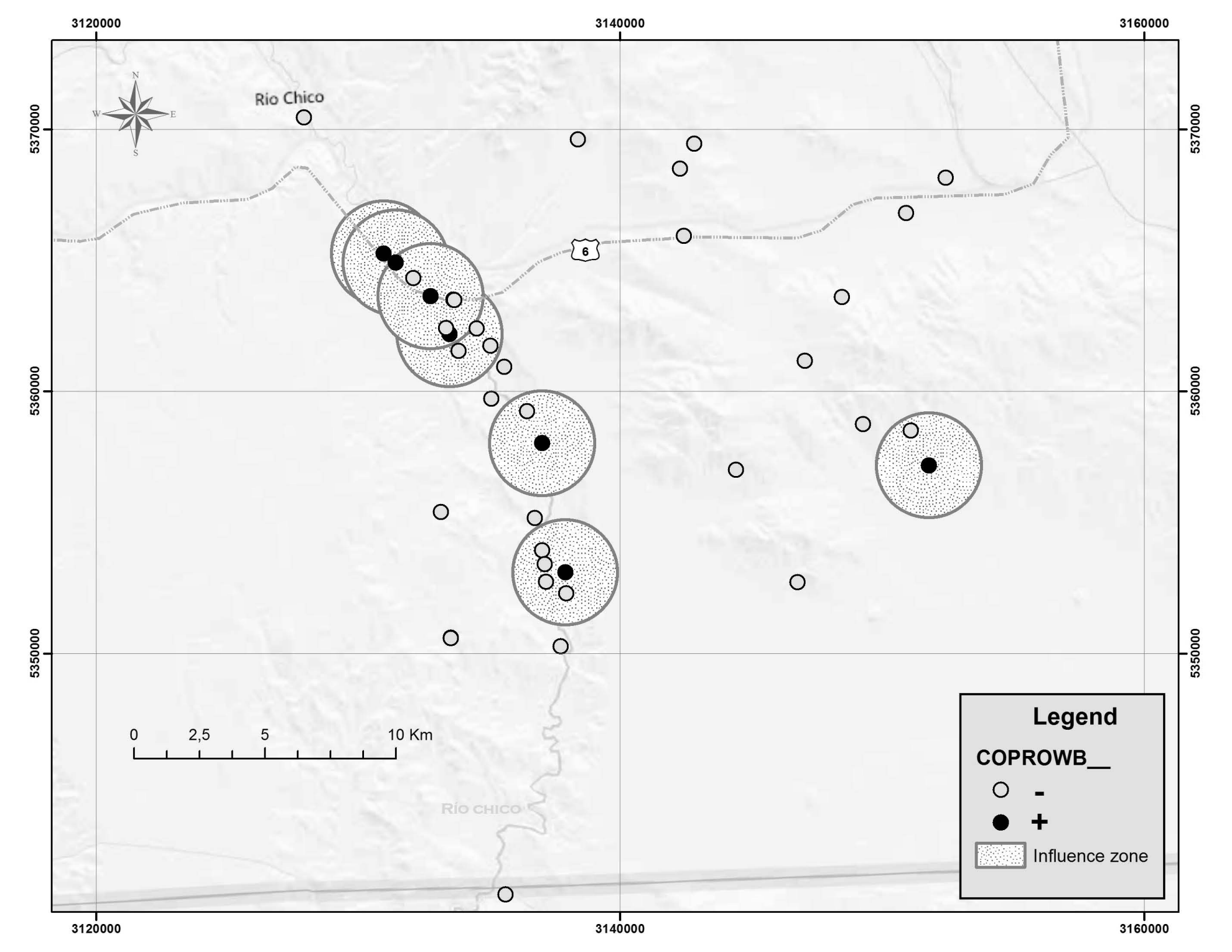
-1.96 - -1.65 Std. Dev.
-1.65 - 1.65 Std. Dev
1.65 - 1.96 Std. Dev.
1.96 - 2.58 Std. Dev.
> 2.58 Std. Dev.
Cluster
Route 6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