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Recombinant S3Pvac-phage anticysticercosis vaccine: Simultaneous protection against cysticercosis and hydatid disease in rural pigs

Julio Morales[a], Aline S. de Aluja[b], Jose Juan Martínez[b], Marisela Hernández[a], Gabriela Rosas[c], Nelly Villalobos[b], Beatriz Hernández[d], Abel Blancas[a], Karen Manoutcharian[a], Goar Gevorkian[a], Jacquelynne Cervantes[a], Alicia Díaz[e], Agnes Fleury[f], Gladis Fragoso[a], Carlos Larralde[a], Edda Sciutto[a,*]

[a] Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de Mexico, Mexico, D.F., C.P. 04510, Mexico
[b] Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de Mexico, Mexico, D.F., C.P. 04510, Mexico
[c] Facultad de Medicina, Universidad Autónoma del Estado de Morelos, Cuernavaca, C.P. 62350, Morelos, Mexico
[d] Facultad de Medicina, Universidad Nacional Autónoma de Mexico, Mexico, D.F., C.P. 04510, Mexico
[e] Centro de Investigación Biomédica de Oriente, Instituto Mexicano del Seguro Social, 4.5 Km, Carretera Atlixco-Metepec, C.P. 74360, Puebla, Mexico
[f] Instituto Nacional de Neurología y Neurocirugía, Insurgentes Sur 3877, México, D.F., 14269, Mexico

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ABSTRACT

This paper provides macroscopic and histological evidence on the statistically significant protective effects of S3Pvac-phage vaccination against porcine cysticercosis and hydatidosis. The study included 391 rustically bred pigs (187 vaccinated and 204 controls). Vaccination significantly reduced the prevalence of cysticercosis by 61.7%. Vaccination also significantly reduced by 56.1% the prevalence of hydatidosis caused by *Echinococcus granulosus* in pigs. The presence of the vaccine epitopes in both cestodes is probably involved in the cross-protection observed. Increased inflammation was found in 5% of cysticerci recovered from controls, versus 24% from vaccinated pigs ($P < 0.01$). Hydatid cysts were non-inflammatory in either group. Vaccination was effective to prevent one single disease, but it failed to prevent the simultaneous infections with both parasites in a same pig. The widening of the S3Pvac-phage vaccine protective repertoire to include hydatidosis is a convenient feature that should reduce the prevalence of two frequent zoonoses that affect rustic porcine breading with a single action. Thus, the costs of two different vaccination programs would be reduced to a single one with significant reduction in both zoonoses.

1. Introduction

Parasite infections by cestodes are frequent health problems widely spread in developed and developing countries. Among the main cestodiases affecting human health and pig production are taeniasis/cysticercosis by *Taenia solium* and hydatid disease by *Echinococcus granulosus* (Sciutto et al., 2000; Moro and Schantz, 2009). In spite of their high impact on human and veterinary health and upon the economy, eradication of both remains a major challenge. This is particularly true in non-developed countries, where cestodes life cycles are firmly established in social, cultural and economic conditions (www.lab.biomedicas.unam.mx/cistimex/s6.html). Intervention programs aimed to improve sanitary conditions, health education, detection and treatment of tapeworm carriers, and the treatment of intermediate hosts have been proposed and success-

* Corresponding author at: Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Circuito Escolar Interior s/n, Ciudad Universitaria Col. Copilco, Mexico, D.F., 04510, Mexico.
Tel.: +52 5556 223 153; fax: +52 5556 223 369.
E-mail address: edda@servidor.unam.mx (E. Sciutto).

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fully tested in focal areas (Sarti, 1997; Sarti et al., 2000; Roman et al., 2000; Boa et al., 2003; Eddi et al., 2003). However, these limited actions are not effective enough to achieve wide and long-term control due to the complex economic and social networks underlying the transmission of cestode diseases (Engels et al., 2003; Pawlowski et al., 2005; Craig and Larrieu, 2006; Larralde and Sciutto, 2006). Increased resistance against the establishment of cestodes larval stage in the intermediate hosts by improving the specific hosts' immune response through vaccination is worth being included as a strategy in control programs. Indeed, extensive reports indicate the effectiveness of different subunit vaccines against cysticercosis and hydatidosis (Siles-Lucas and Gottstein, 2003; Siles-Lucas et al., 2008; Lightowlers et al., 1996). We recently reported that S3Pvac-phage anti-cysticercosis vaccine reduced cysticercosis prevalence by 54% and the number of cysticerci established by 87% in 331 pigs in a field trial performed in rural Mexico (Morales et al., 2008b). Based on these successful results, S3Pvac-phage vaccine has been included in an ongoing control program applied in wider geographic areas of Mexico since 2009 (Olguín, 2010).

On the other hand, as the research progressed we realized that at least two of the three peptides of the S3Pvac vaccine were shared by other cestodes, i.e. *E. granulosus*, *Echinococcus multilocularis* and *Taenia saginata* (Rassy et al., 2010). This finding makes feasible that S3Pvac-phage could increase not only resistance against *T. solium* cysticercosis but also resistance against hydatidosis. This would be particularly relevant considering that the transmission of both *T. solium* (Aluja, 2008) and *E. granulosus* (Moro and Schantz, 2009) is promoting by the common practice of rearing free-ranging pig in rural areas of non-developed countries (Aluja, 2008), where poor sanitary conditions prevailed. Both cestodiases in pigs cause economic losses, pose a risk for people consuming pork, and are relevant in transmission of these parasitoses.

The objective of this study is to evaluate the effects of S3Pvac-phage vaccination upon the prevalence of naturally acquired cysticercosis and hydatidosis in 391 pigs from Central Mexico. Moreover, the effect of vaccination on the microscopic appearance of established *T. solium* and *E. granulosus* cysts was histologically examined.

2. Materials and methods

2.1. S3Pvac phage vaccination program

Peptide-phage recombinants KETc7 (FK7), GK1 (FGK1), KETc1 (FKETc1), and KETc12 (FKETc12) were prepared as previously reported (Manoutcharian et al., 2004) and used together as the anti-cysticercosis vaccine (S3Pvac-phage).

Each pig-dose of S3Pvac-phage vaccine contained 10^{12} formaldehyde-heat-inactivated phage particles of each recombinant in 2 ml. Vaccination program was carried out from April 2004 to July 2006, and included a total of 1047 pigs from 16 rural communities of Huautla, Morelos, México. In these communities, 13.3% pig cysticercosis prevalence was determined before applying the vaccination program (Morales et al., 2008a). Half of the piglets in each litter randomly selected received two subcutaneous injections of S3Pvac-phage in the ear base at 3–4 months of age and one month later; the other half of the litter received saline as placebo, as previously described and reported in detail (Morales et al., 2008b).

2.2. Follow-up of vaccination trial

At 5–27 months of age, when owners slaughtered pigs for consumption, cysticercosis was diagnosed at necropsy by dissecting masseters, tongue, diaphragm, heart and liver, in search of visible cysticerci, by making parallel scalpel slices 5 mm-wide. The number of cysticerci found in each pig was recorded, and the owners were instructed not to eat the meat if pigs were infected. A total of 391 necropsies were performed in 187 vaccinated and 204 non-vaccinated pigs, 60 pigs more than those included in the previous report (Morales et al., 2008a). During necropsies, liver and peritoneal cavities were visually inspected in search of hydatid cysts or other visceral helminths. Lesions compatible with parasite infection and all suspicious tissue were collected for histological analysis.

2.3. Histological studies

T. solium and *E. granulosus* cysts samples taken at necropsy were fixed in 10% buffered formalin, processed for paraffin sections and stained with hematoxylin–eosin. Hydatid cysts and host tissues surrounding them were analyzed for microscopic inflammation signs. A significant representative number of 1120 and 120 cysticerci of the total number of cysticerci, 1459 and 162 in control vaccinated pigs respectively were classified in five histological grades, according to developmental stage and degree of inflammation, as previously reported by Aluja and Vargas (1988), with minor modifications. Vesicular cysticerci were considered viable, and showed either no or slight histological changes with low inflammatory immune response. These cysticerci correspond to metacestodes, classified as grades 0, 1 and 2 according to Aluja and Vargas (1988). Low viability, colloidal or hyaline cysticerci showed intermediate inflammation and were classified as grades 3 and 4 by Aluja and Vargas (1988). These parasites are surrounded by inflammatory cells, with eosinophils dominating the infiltrate. Caseous cysticerci were accompanied by abundant inflammatory infiltrate and necrotic debris, in which hooks and calcareous corpuscles were distinguished. Calcified cysticerci were almost totally destroyed and their structures substituted by calcified material, with or without a surrounding chronic inflammatory response from the host. These lesions were considered as grades 5 and 6 by Aluja and Vargas (1988) (Fig. 2).

2.4. Immunolocation of S3Pvac peptides

2.4.1. Rabbit antibodies

To immune-localize vaccine peptides in anatomical structures of the parasites under study, specific rabbit anti-KETc1, anti-KETc12 and anti-GK1 antisera were prepared by immunization of female New Zealand white rabbits, one rabbit for each peptide. Rabbits were bled before being subcutaneously immunized with 100 μg of each synthetic