Archives of Razi Institute, Vol. 63, No. 2, December (2008) 29-34

Experimental vaccination of sheep against hydatid cyst using EG95 recombinant vaccine

Paykari [*1], H., Heath[2], D.D., Dalimi[1], A.H., Karimi[1], Gh.R., Motamedi[1], Gh. R.
1. Department of Parasitology, Razi vaccine & Serum Research Institute,Karaj, Iran
2. AgResearch, Wallacevill Animal Research Center, New Zealand

Received 16 Feb 2007; accepted 05 Nov 2008

ABSTRACT

Vaccination of livestock with effective vaccine could be one of the control methods for hydatid cyst. In this study a new recombinant vaccine (Eg95) from New Zealand was prepared and used. Thirty health sheep from the same race and identical age and sex were selected. There was no history of any past vaccination or disease in selected animals. Animals were randomly categorized to four groups. One group including ten sheep received two times vaccination and each time 50 microgram with two weeks interval. Another group (10 sheep) received physiological saline without vaccine. Five weeks after second vaccination both groups were challenged with 3500 freshly *Echinococcus granulosus* (Iran isolate, dog/sheep cycle) eggs intraruminally. Animals, in third and fourth groups, received vaccine and physiological saline respectively. Third and fourth groups did not challenge and kept for vaccine adverse effects or natural infection control. Anti-Eg95 antibody titer was evaluated with ELISA before and two weeks after each vaccination. Optical density (OD) rates for all groups were less than 0.1 before vaccination. A 40 fold increase in OD rates of vaccinated groups was seen 2 weeks after second vaccination. Animals under the study were fully surveyed. Results indicated 98% protection in vaccinated group when they were necropsied 12 months after challenge .The EG95 vaccine can be produced on an industrial scale and can be further use for clinical trial in Iran.

Keywords: Recombinant vaccine, *Echinococcus granulosus* eggs, Hydatid cyst, Sheep

INTRODUCTION

Echinococcusis/hydatidosis is one of the most important zoonotic diseases prevalent in different parts of Iran (Mobedi & Dalimi 1994). Various studies have indicated that hydatid cyst is commonly found in sheep, cattle's, goats and camels as intermediated hosts and widespread recovery of *Echinococcus granulosus* has been reported from dogs, jackals and wolves as final hosts throughout Iran (Mobedi *et al* 1970, Eslami 1990, Mobedi & Dalimi 1994, Oryan *et al* 1994, Dalimi *et al* 2002, Dalimi *et al* 2004). Furthermore, human cases are regularly reported from medical centers in different parts of Iran (Sadjjadi 2006). Hydatid cyst has been reported from all countries in Middle East as well as Arabic North Africa (Sadjjadi 2006). There are at least 3 strains of *E. granulosus* in the Middle East: The sheep strain

*Author for correspondence. E-mail:h.paykari@rvsri.ir

(G1) which exists in sheep, goat, cattle, buffaloes, camels and pigs; the horse strain (G4) based on hook morphology, which exists in horses and donkeys and the camel strain (G6) which is specific for camel and is distinct from sheep strain that may infect camels (Sadjjadi, 2006). Two strains of *E. granulosus* (G1 and G6) were reported in Iran (Zhang *et al* 1998). Cysts slowly grow in grazing animals that have eaten eggs of tapeworm, and become infective to dogs after 2-5 years (Heath *et al* 2003). The life cycle is completed when final hosts eat infective cysts. Control of transmission of disease relies on education of the public to prevent access of dogs, to the offal of infected intermediated hosts, and a regular treatment of dogs with a cestocidal anthelmintic. Control campaigns based on those methods can be successful; however, they typically require intensive effort over several years. Any breakdown in infection control in dogs may lead to substantial numbers of new infection in intermediated hosts, providing a continuing source of infection for transmission of parasite (Lightowlers *et al* 1999). Since sheep play a major role in transmission of the parasite globally, this paper describes laboratory testing of Eg95 vaccine in sheep against hydatid cyst in Iran.

MATERIALS AND METHODS

Vaccine. Vaccine has partially purified extract of killed genetically modified *E. coli* K12, which has expressed a fusion protein. The *E. granulosus* antigen, known as Eg95 (23-25 KD) is fused to Gluthathion S-Transferase (GST) from *S. japonicum.* The vaccine protein is obtained from *E. granulosus* mRNA expressed in bacteria using recombinant DNA techniques. The adjuvant is Quil A that stimulates CMI responses (Lightowlers *et al* 1996 and Heath *et al* 2003). The vaccine was obtained from Wallaceville Animal Research Center, New Zealand.

Eggs. Infected livers and lungs of sheep were obtained from abattoir and dogs were infected with eating of these organs. Gravid *E. granulosus* worms were obtained from experimentally infected dogs and eggs were prepared from them.

Test groups. 30 male sheep of 6-12 months were obtained from Razi Institute farm. Animals were examined to rule out parasitic diseases and other infection diseases. Sheep's were divided into 4 groups**:**

1. Test group: 10 sheep were vaccinated and challenged.
2. Control group: 10 sheep were not vaccinated and were challenged.
3. Control group: 5 sheep were vaccinated but were not challenged.
4. Control group: 5 sheep were neither vaccinated nor challenged.

Vaccination protocol. The final dose of vaccine was 1 ml with 50 µg Ag and 1mg adjuvant. The injection was administered subcutaneously to the anterior right- of the neck region. Second vaccination was conducted after four weeks with the same procedure. Before vaccination and two weeks after each vaccination, blood samples were collected from all animals for detection of anti-Eg95 IgG titer with ELISA.

ELISA. It was performed as previously described by (Heath *et al* 2003): Several numbers of plates were pre-coated with 100 µL of 1:1000 ELISA-Ag in carbonate buffer. Wells were blocked with 200 µL blocking buffer and incubated at room temperature for 60 min. All plates were washed and then stored at -20 ºC until required. 100 µL of diluted Sera (1:200 in blocking buff.) were added to each well and incubated for 90 min at room temperature. Positive control, negative control and standard sera were added to each plate. Wells were washed and 100 µL anti-sheep IgG-HRP conjugate (Sigma) was added (1:2500) and incubated 90 minutes at RT. Plates were washed and 100 µL prepared OPD was added to each well and

incubated 20 minutes at RT. 5. The reaction was stopped with 50 µL 1M sulphuric acid and read at 490 nm with ELISA reader.

Challenge method. Groups 1 and 2 were challenged with 3500 eggs of *E. granulosus* (Iran isolate, dog/sheep cycle) four weeks after second vaccination. All animals were monitored and controlled for more than one year. All sheep were slaughtered 12 months after challenge. Liver, lung, heart and kidneys of all sheep were examined for the presence of hydatid cyst.

RESULTS

ELISA was performed on sera from control and vaccinated sheep and results are showed in Figure 1.

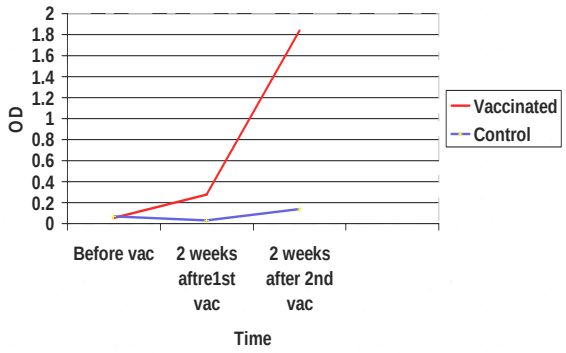

Figure 1. Mean of optical density (OD in ELISA against EG95 in vaccinated sheep and control group.

Anti-Eg95 IgG was increased in vaccinated group after first vaccination but a high IgG increasing was observed two weeks after second vaccination in vaccinated sheep. These results show two vaccinations with 4 weeks interval are necessary to induce enough antibodies. All sera of vaccinated sheep had an OD >1.5 two weeks after second vaccination at 1:200 dilution, while OD of these sera was less than 0.1 before vaccination. All sheep were necropsied 12 months after second vaccination and results showed non-immunized animals had a mean of 130.89 *E. granulosus* cysts, while vaccinated sheep were highly protected (98% protection, Table 1). 60% of vaccinated animals did not have any hydatid cyst and 20% of them only had 1 hydatid cyst in their lungs. 10 hydatid cysts were only seen in one vaccinated sheep and 7 hydatid cysts were seen in another one of vaccinated sheep. Minimum and maximum diameters of cysts in all groups were 2 and 12 millimeter respectively. Diameters of cysts were not different between groups.

Table1. Mean of hydatid cyst number in vaccinated sheep with EG95 and control group.

Group	No of Sheep	Hydatid cyst		Min	Max
		Mean	S.D		
Vaccinated	10	2.11*	3.72	0	10
Control	10	130.89	46.45	54	182

*P<0.001

DISCUSSION

Hydatid disease is a parasitic infection caused by cestodes of the genus *Echinococcus*. The most important and widespread of these parasites is *E. granulosus*, which causes cystic hydatid disease. Surgery is the standard form of treatment; drugs are of limited value. Human infections are most common in the Mediterranean region, sub-Saharan Africa, Russia, China and South America (Shantz *et al* 1995, Gemmell *et al* 1987, Ekert *et al* 1981 & Matossian *et al* 1977). Control programs for hydatid disease have been, or are being, undertaken either nationally or in regional areas. These programs rely on public education, restrictions on livestock slaughtering and control measures in dogs. Despite substantial efforts to reduce transmission of the parasite,

hydatid disease remains a serious cause of human morbidity in many parts of the world (Lightowlers *et al* 1999). Recently, a vaccine has been developed as a new tool to assist with control of hydatid disease. The EG95 vaccine has been developed to prevent infection with *E. granulosus* in intermediate hosts (Heath & Lawrence, 1996, Lightowlers *et al* 1996 & Lightowlers *et al* 1999). The vaccine contains a protein which occurs in the parasite egg and early developmental stages. Parasites attempting to establish an infection in a vaccinated host are killed by the immune responses induced by the vaccine (Heath *et al* 2003). Immunization with the protein, termed EG95, has been shown to prevent hydatid infection in sheep (Lightowlers *et al* 1996 & Lightowlers *et al* 1999). The mechanism by which the vaccine has its effect is through antibody and complement mediated lysis of the parasite oncosphere early in the establishment of a new infection. Protection can be demonstrated either following challenge infection or by demonstrating lysis of the parasite in vitro in the presence of specific serum antibodies to the vaccine (Heath *et al* 2003). A study indicates that the major part of the immune response induced by EG95 vaccination is directed against conformational epitopes and that the host-protective epitopes are conformationa (Woollard *et al* 2001). A large scale trial has confirmed safety and efficacy of this vaccine (Heath *et al* 2003). One study has shown that co-delivery of cytokine genes can modulate the immune response generated by a DNA prim-recombinant protein boost vaccination strategy (Scheerlinck *et al* 2001). The characteristics of the immunity stimulated by the EG95 vaccine are summarized as (Lightowlers *et al* 1999): Two immunizations stimulate greater than 95% protection against hydatid infection in sheep. These results are similar as our study. More than 50% of vaccinated animals have no viable hydatid cysts after challenge infection with *E. granulosus*. Immunity persists for at least a full year after two immunizations with the vaccine. Approximately 80% immunity is induced in sheep after a single injection. Solid immunity is transferred with colostral antibody from a vaccinated dam to neonatal offspring. The vaccine has been shown to be similarly effective in trials carried out in Argentina, Australia, China and New Zealand. The vaccine has been shown to be effective in other animal hosts of *E. granulosus*, including goats and cattle (Heath *et al* 2003). One study in china shows the Eg95 gene is expressed in oncospheres, protoscoleces, and immature and mature adult worms, and the Eg95 gene family was shown to comprise two basic sequence types. Very limited sequence variation was evident in the EG95 protein from oncospheres. This high degree of sequence conservation predicts that the vaccine will continue to be effective in China and elsewhere (Zhang *et al* 2003). The amino acid substitutions of EG95 in the G6/G7 genotypes may affect the antigenicity/efficacy of the EG95 recombinant antigen against parasites of these genotypes. These findings indicate that characterization of EG95 gene family members in other strains/isolates of *E. granulosus* may provide valuable information about the potential for the EG95 hydatid vaccine to be effective against *E. granulosus* strains other than the G1 genotype (Chow *et al* 2008). The EG95 vaccine can be produced on an industrial scale and it is being developed further for widespread use in livestock animals. It would be an advantage if the vaccine was able to be mixed with other vaccines. Preliminary work indicates that the vaccine will work effectively in the presence of an aluminium hydroxide-adjuvanted clostridial vaccine of five

strains in one. The aluminium hydroxide actually enhances the effect of the Quil A on both the clostridial and the hydatid vaccine, despite aluminum hydroxide not being a useful primary adjuvant for the hydatid vaccine. The disadvantage of this combination is that the aluminum hydroxide vaccine cannot be freeze-dried. The shelf-life of a liquid hydatid vaccine which incorporates a clostridial vaccine needs to be determined. The hydatid vaccine can also be incorporated with other injections, including injectable anthelmintic. The formulation that a particular country wishes to use will require the appropriate testing and registration in that country (Heath *et al* 2003). Reduction in the prevalence of the disease in farm animals would be expected to have an important impact on the potential for transmission of the parasite by dogs and, indirectly, reduce the number of new human infections. Use of the vaccine together with dog control measures and public education may increase the effectiveness of hydatid control campaigns and reduce the period of time over which control measures are required. This would have a substantial impact on the incidence of human hydatid infections.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J. and Miller, W. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389-3402.

Chow, C., Gauci, C.G., Vural, G., Jenkins, J.J., Heath, D.D., Rosenzvit, M.C., Harandi, M.F. and Lightowlers, M.W. (2008). *Echinococcus granulosus*: Variability of the host-protective EG95vaccine antigen in G6 and G7 genotypic variants. *Experimental Parasitology* 119: 499–505.

Dalimi, A., Malaki, M. and Motamedi, G.H. (2004). The potential role of golden jackals and sheep in Echinococcosis / Hydatidosis life cycle in Ilam province, West Iran, *Pajouhesh & sazandegi*, 63: 6-29.

Dalimi, A., Motamedi, G.H., Hosseini, M. and Mohammadian, B. (2002). Echinococcosis/ hydatidosis in Western Iran. *Veterinary Parasitology* 105: 161-171.

Eckert,J., Gemmell, M.A., and Soulsby, E.J.L. (1981) FAO/UNEP/WHO Guidelines for the surveillance, prevention and control of echinococcosis/hydatidosis, *World Health Organizatio*n, Geneva.

Eslami, A. (1990). Cestoda In*: Veterinary Helminthology* (vol. 2) Pp: 124-125. Tehran University (in Persian).

Gemmell, M.A., Lawson, J.R. & Roberts, M.G. (1987). towards global control of cystic and alveolar hydatid diseases. *Parasitology Today* 3:144-151.

Heath, D.D. and Lawrence, S.B. (1996). Antigenic polypeptides of *Echinococcus granulosus* oncospheres and definition of protective molecules. *Parasite Immunology* 18: 347-357.

Heath, D.D., Jensen, M.W. and Lightowlers, M.W. (2003). Progress in control of hydatidosis using vaccination- a review of formulation and delivery of the vaccine for practical use in control programmes, *Acta Tropica* 85: 133-143.

Lightowlers, M.W., Lawrence, S.B., Gauci, C.G., Young, J., Ralston, M.J., Maas, D. and Heath, D.D. (1996). Vaccination against hydatidosis using a defined recombinant antigen. *Parasite Immunology* 18: 457-462.

Lightowlers, M.W., Jensen, O., Fernandez, E., Iriarte, J.A., Woollard, D.J., Gauci, C.G., Jenkins, D.J. and Heath, D.D. (1999). Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccin e in sheep. *International Journal of Parasitology* 29: 531-534.

Lightowlers, M.W. and Heath, D.D. (2004). Immunity and vaccine control of *Echinococcus granulosus* infection in animal intermediate hosts, *Parassitologia* 46: 27-31.

Matossian, R.M., Rickard, M.D. and Smyth, J.D. (1977). Hydatidosis: a global problem of increasing importance. *Bulletin WHO* 55: 499-507.

Mobedi, I. and Dalimi, A. (1994). *Epidemiology of hydatid cyst in Iran and world*. Moghadam Publication (in Persian).

Mobedi, I., Madadi, R.A. and Arfaa, F. (1970). Camel "Camelus dromedarios" as intermediate host of *Echinococcus granulosus* in Iran. *Journal parasitology* 56: 1255.

Oryan, A., Moghadar, N. and Guar, S.N.S. (1994). Metacestodes of sheep with special references to their epidemiologicals tatus, pathogenesis and economics

implications in Fars province, Iran. *Veterinary Parasitology* 51: 23 1-240.

Sadjjadi, S.M. (2006). Present situation of echinococcosis in the Middle East and Arabic North Africa, *Parasitology International* 55: S197-S202.

Schantz, P.M., Chai, J., Craig, P.S., Eckert, J., Jenkins, D.J., Macpherson, C.N.L. and Thakur, A. (1995). *Epidemiology and control of hydatid disease. In Echinococcus and Hydatid Disease*, RCA Thompson & AJ Lymbery Pp: 233-331; CAB International, Wallingford.

Scheerlinck,.J.P.Y., Casey, G., McWaters, P. , Kelly, J., Woollard, D. , Lightowlers, M.W., Tennent, J.M. and Chaplin, P.J. (2001). The immune response to a DNA vaccine can be modulated by co-delivery of cytokine genes using a DNA prime-protein boost strategy.

Zhang, W., Li, J., You, H., Zhang, Z., Turson, G., Loukas, A., and McManous, D.P. (2003). Short report: *Echinococcus granulosus from* Xinjiang, PR CHINA: cDNAs encoding the EG95 vaccine antigen are expressed in different life cycle stages and are *Vaccine*, 19, 4053–4060conserved in the oncospher, *American Journal of Tropical Medicine Hygeine* 68(1): 40-43.

Woollard, D.J., Gauci, C.G., Heath, D.W. and Lightowlers, M.W. (2001). Protection against hydatid disease induced with the EG95 vaccine is associated with conformational epitopes, *Vaccine* 19: 498-507.

Zhang, L., Eslami, A., Hosseini, S.H. and McManus, D.P. (1998) Indication of presence of two distinct strains of *Echinococcus granulosus* in Iran by mitochondrial DNA markers. *American Journal of Tropical Medicine Hygeine* 59: 171-174.