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Immunization of lambs with whole body *Echinococcus granulosus*

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Summary

We conducted this study to determine the level of immunity after vaccination of lambs with whole body *Echinococcus granulosus*. To do so, 200 mature *E. granulosus* parasites, which were kept in 10% formaline for 8 months, were obtained from the Department of Parasitology, School of Veterinary Medicine, Ferdowsi University of Mashhad. The soluble protein of the parasite was prepared. The sample was homogenized in a blender, sonicated on ice and then centrifugated for 15 min at 10,000 g. Final yield was kept at -20ºC until used. Eight 4–6-month-old lambs of mixed sex, were divided into 2 equal groups; each lamb in the test group was vaccinated subcutaneously in the neck with 2 ml of the vaccine (1 mg of whole body of *E. granulosus* protein dissolved in 1 ml of PBS plus 1 ml of Freund's complete adjuvant (FCA)). The control lambs were vaccinated only with adjuvant in PBS. Lambs were re-vaccinated four weeks after the first vaccination with the same preparation except that FCA was replaced by Freund's incomplete adjuvant (FIA). Three weeks later, each lamb was administered a challenge infection dose of 2000 protoscolices intraperitoneally and 10 adult *E. granulosus*. After 7 months, all lambs were killed and examined for hydatid cysts. We found two cysts in the liver and and one in the lung of only one of the vaccinated lambs. The number of cysts in vaccinated lambs were significantly lower than that in the control group ($P<0.001$). This means that the protective immunity in lambs with whole body of *E. granulosus* was approximately 90%.

Key words: *Echinococcus granulosus*, Whole body, Vaccination, Lamb

Introduction

Hydatidosis is one of the most important zoonoses in Iran. The importance and prevalence of this disease has been highlighted by several surveys. Although many species of domestic livestock and herbivorous wildlife species are potential hosts for *E. granulosus*, sheep play a major role in transmission of the parasite globally (Lightowlers *et al*., 1999). Recent evidence points out that cystic echinococcosis is a public health problem of increasing concern in a number of countries where controlling programes have been reduced due to economic problems and lack of resources, or have yet to be fully investigated (Eckert *et al*., 2001). The intermediate hosts of *E. granulosus* are long-lived and infection by eggs provokes a high degree of protective immunity, a characteristic that has been used for the development of a highly effective vaccine (Lightowlers *et al*., 1996). Hydatid disease is common throughout the world where pastoralism is practised, and is particularly common in population with limited education and hygiene. In some parts of the world, some investigators have developed some effective vaccines against the disease. Most grazing animals are already vaccinated against viral or bacterial diseases, and so a vaccine against a parasitic disease can fit into normal farm practice (Heath *et al*., 2003). A vaccine to protect grazing animals against echinococcosis is an additional control method that focuses on grazing animals instead of the dog. Ongoing investigations will shed light on the biological roles played by the proteins within the parasites and the mechanism by which they make the parasites vulnerable to vaccine-induced immune responses (Light-

owlers *et al*., 2003). The extraordinary effectiveness of the hydatid vaccine in the parasite's natural animal hosts singles this vaccine out as having perhaps the greatest potential for development of the first effective human vaccine against a parasitic disease (Lightowlers, 2002). In the current study, the presence of hydatid cysts and also protective immunity after vaccination of lambs with whole body *E. granulosus* antigen is investigated.

Materials and Methods

Preparation of antigen

Two hundred mature *E. granulosus* parasite were obtained from the Department of Parasitology, School of Veterinary Medicine, Ferdowsi University of Mashhad. These parasites were kept in 10% formaline for 8 months. They were washed three times with Hanks solution. After washing with phosphate buffer saline (PBS) (pH = 7.3), they were kept in PBS at -20ºC. The soluble protein of mature *E. granulosus* was prepared by freezing-thawing in liquid nitrogen and 42ºC for three times. The sample then was homogenized in a blender; sonicated at 110 V, 170 W ultrasonic disintegrator (Hielscher, Germany) for 3×15 sec on ice; and centrifugated at 10,000 g for 15 min. Finally, the sample was filtrated with 0.22 μm filter. The protein concentration was measured by Bradford (1976) method. It was kept at -20ºC until used.

Vaccination

Eight 4–6-month-old lambs of mixed sex, were divided into 2 equal groups. Each lamb in group 1 (test) was vaccinated subcutaneously in the neck with 2 ml of the vaccine (1 mg of whole body *E. granulosus* protein dissolved in 1 ml of PBS plus 1 ml of Freund's complete adjuvant (FCA)). The control lambs were vaccinated with a total volume of 2 ml of adjuvant in PBS. Lambs were re-vaccinated four weeks after the first dose with the same preparation except that FCA was replaced by Freund's incomplete adjuvant (FIA).

Challenge

Three weeks after the second vaccination, each lamb was administered a challenge infection dose of 2000 protoscolices intraperitoneally and 10 adult *E. granulosus* worms. Worms were injected into the rumen via a 16 G 10-cm needle fitted with a disposable three-way stopcock. Prior to injection of worms, a small amount of water was injected into the rumen via a second syringe connected to the stopcock. The fluid was immediately withdrawn to see the rumen contents so that we could verify the correct placement of the needle. After injection of worms, the needle was flushed with approximately 20 ml water via a second syringe also fitted to the stopcock (Lightowlers *et al*., 1999). This method ensured the delivery of the same dose of *E. granulosus* into the rumen of each lamb and prevented any possibility of contamination of the operators with *E. granulosus*.

Measurement of protective immunity

The protective immunity (PI%) in lambs was defined by Dempster *et al*., (1995) as follows:

$$\text{PI}(\%) = 1 - \frac{\text{Average of cysts in test group}}{\text{Average of cysts in control group}} \times 100$$

Seven months after the experimental infection, all lambs were slaughtered and examined for the presence of hydatid cysts. The carcasses were dressed and examined superficially. The heart and kidneys were sliced and the omentum and spleen were also examined. The liver and lungs were examined extensively; the liver was sliced at intervals of approximately 2 cm. The lungs were sliced at intervals of approximately 4–5 cm and palpated.

Statistical analysis

The results of this experiment was analysed by Student's t-test.

Results

In all control lambs there were a lot of cysts in liver and lungs after seven months of challenge. The number of hydatid cysts in each lamb of the control group were more than 10 cysts. In the control lambs, the percentage of hydatid cysts in liver and lung was about 52.5 and 47.5, respectively. on the other hand, three of the vaccinated lambs,

had no hydatid cysts. We observed two cysts in the liver and one cyst in the lung of only one of the vaccinated lambs. In vaccinated lambs with whole body *E. granulosus*, hydatid cysts were either not observed, or in comparison with the control group, few smaller cysts were observed. The mean size of hydatid cysts in the control group were 9.5 mm; whereas, it was 2.5 in the vaccinated lambs. The number of cysts in vaccinated lambs were significantly lower than that in the control group ($P<0.001$). The PI%, as defined earlier, with adult *E. granulosus* was approximately 90%.

Discussion

We used the crude antigen of *E. granulosus* for immunization of lambs. The protective immunity of the antigen was very high. Our results were in keeping with those of the studies that have been done either with the native oncosphere antigen (Heath and Lawrence, 1996) or the EG95 recombinant antigen (Lightowlers *et al*., 1996). Three experimental vaccine trials in Australia and Argentina against hydatid disease have been undertaken in sheep using EG95 protein which was expressed in *E. coli*. Based on the number of viable cysts in the control and vaccinated sheep, the vaccine was 96% effective (Lightowlers *et al*., 1999). Serum from sheep vaccinated with the EG95 recombinant antigen could induce in vitro antibody-dependent, complement-mediated lysis of oncospheres, just similar to that of the native molecule. Both the EG95 recombinant protein and its native oncosphere equivalent appear to be resistant to denaturation (Heath and Lawrence, 1996). Lightowlers and Heath (2004) developed a vaccine based on EG95 to prevent hydatid infection in animal intermediate hosts of *E. granulosus*. The vaccine comprises a single recombinant oncosphere antigen and the adjuvant Quil A. It induces complement-fixing antibodies that kill the invading oncosphere in the early stage of the infection. It has been reported that lambs which received two immunizations with oncospheral antigens were protected against an oral challenge infection with *E. granulosus* eggs. While all control sheep developed numerous hydatid cysts as a result of the challenge infection, seven (88%) of the eight vaccinated sheep were completely protected by the immunization (Osbon and Heath, 1982). Protective immunity in buffaloes induced by egg and oncosphere of *E. granulosus* antigens were 76.7 and 83.5%, respectively (Navidpour *et al*., 2003). They reported that the egg and oncosphere are the most potent sources of protective antigens for immunity of buffaloes against hydatid cyst. A vaccine based on *E. granulosus* antigen, protected sheep from infection with *E. granulosus.* A defined antigen vaccine has been developed which can prevent hydatid infection in sheep. The vaccine has been shown to be highly effective in animal trials, with almost complete immunity persisting for more than one year after vaccination (Lightowlers *et al*., 1999). Use of the vaccine in livestock may decrease transmission of the parasite and indirectly, reduce the incidence of infections in humans. In some regions, vaccination of animals or other controlling measures are unlikely to be applicable. In these areas, direct vaccination of humans against hydatid infection may be the only practical option for disease prevention (Heath *et al*., 2003). A vaccine candidate for the intermediate host has been tested in sheep, showing a high degree of protection (Lightowlers *et al*., 1996; Woollard *et al*., 1998). Results in these studies showed that crude antigen of *E. granulosus* could be a good candidate for vaccination of intermediate host.

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