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Source: *The Journal of Parasitology*, Vol. 67, No. 6 (Dec., 1981), pp. 797-799

Published by: [The American Society of Parasitologists](#)

Stable URL: <http://www.jstor.org/stable/3280702>

Accessed: 04/05/2011 22:45

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RESISTANCE TO *ECHINOCOCCUS GRANULOSUS* INFECTION IN LAMBS

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ABSTRACT: A high level of resistance to oral infection with *Echinococcus granulosus* eggs was stimulated in lambs by two or more subcutaneous injections of oncospheres given 14 days apart. The degree of resistance was significantly higher than that resulting from a single injection. Resistance was apparently stimulated by the activated oncosphere or a stage of cyst development prior to 14 days of age. Studies on oncospheres cultured in vitro in sera collected from animals during immunization or after oral challenge showed that most cysts were killed before 7 days of culture had elapsed. This confirmed the observation that resistance was stimulated by an early stage of cyst development. The in vitro test also showed that two injections of oncospheres resulted in a marked increase in the lethal effects of serum. These lethal effects decreased with time, providing circumstantial evidence that the high degree of resistance stimulated by two or more injections may only be transient.

Resistance to the establishment of an *Echinococcus granulosus* infection following a single exposure to homologous eggs or oncospheres has been reported on a number of occasions. Sweatman et al. (1963) found that approximately 70% of a challenge infection was prevented from establishing following oral infections of from 10 to 10,000 eggs given 9 mo earlier. A similar degree of protection was obtained more recently by Yarulin (1968) and Aminzhanov (1976). Gemmell (1966) achieved 91.2% rejection of a challenge infection given to lambs 3 mo after immunization by intramuscular injection of oncospheres. Immunizing doses of from 1,000 to 50,000 oncospheres did not significantly alter the degree of resistance, and in most cases cysts grew at the injection site.

Heath et al. (1979) achieved apparently complete resistance to establishment of an oral challenge of *E. granulosus* after an oral infection, followed by two intramuscular injections of eggs at monthly intervals prior to the challenge. In the experiment reported here, some parameters of the induction of resistance were examined more closely, with a view to the development of a vaccination procedure for sheep.

MATERIALS AND METHODS

Forty Romney lambs were reared on pasture free of taeniid eggs, and when 3 mo old were randomly allocated into five test groups (5 lambs per group)

and two control groups (one group of 5 and one group of 10). In the five test groups oncospheres were injected subcutaneously into lambs according to the schedule shown in Table I. Each injection consisted of 2,000 oncospheres of *E. granulosus*, prepared according to the method of Heath and Smyth (1970). Repeat injections were administered at different sites (the order was as follows: left thoracic, right thoracic, left upper hind leg, right upper hind leg, center mid back). All groups, except the unchallenged controls, were dosed with 2,000 freshly collected *E. granulosus* eggs 14 days after their final injection. Times of injection were arranged so that all animals could be challenged on the same day.

At the time of each injection, the injected lambs and the control lambs were bled to provide sera for an in vitro examination of the ability of the sera to kill developing *E. granulosus* cysts. The technique used was that of Heath and Lawrence (1981). Further bleedings of all sheep were made at the time of oral challenge with eggs, at 14 and 56 days after challenge, and at necropsy 6 mo after challenge.

At necropsy, the lambs were skinned and the injection sites were inspected for evidence of developing cysts. Then, the livers and lungs were finely sliced, and the slices palpated (wearing surgical gloves), to determine the number of *E. granulosus* cysts developing. With the immunized lambs, all cysts were opened to determine whether there was a fluid-filled cavity. Cysts were classed as dead if the lesion was solid, with no cavity observable macroscopically. Fifty cysts in the liver and 50 in the lung of each control lamb were also sliced open. The outer diameter and diameter of the lumen of each cyst was measured.

RESULTS

Almost all (22/25) lambs from Groups 1 to 5 had masses of cysts developing at the first injection site. At necropsy the diameter of cysts within these masses ranged from 1 to 13.5 mm, with a mean of approximately 5 mm.

Received 16 January 1981; revised 22 April 1981; accepted 12 July 1981.

TABLE I. Number and size of *Echinococcus granulosus* cysts developing in injected or control lambs following oral infection with 2,000 eggs.

Treatment	Number of days prior to oral challenge when injections were made					Number of cysts in individual lambs						Mean diameter of cysts (mm)								
	70	56	42	28	14						Liver		Lung							
						Mean					Outer Lumen	Outer Lumen	Outer Lumen	Outer Lumen						
Challenged control						750	633	680	692	375	541	2.7	1.9	4.2	3.3					
Injected Group 1					+	151	644	344	508	638	18	0	3	32	37	18*	2.8	1.9	3.5	2.2
Injected Group 2				+	+	0	0	0	0	0	0	0	0	0	0	0	—	—	—	—
Injected Group 3			+	+	+	0	0	5	0	0	1.0	2.0	0.0	3.0	0.0	—	—	—	—	
Injected Group 4		+	+	+	+	0	0	0	1	0	0.2	—	—	6.0	3.5	—	—	—	—	
Injected Group 5	+	+	+	+	+	0	0	0	0	0	0	—	—	—	—	—	—	—	—	
Unchallenged control						0	0	0	0	0	0	—	—	—	—	—	—	—	—	

* Group 1 significantly different ($P < 0.01$) from Groups 2 to 5 combined, using Mann-Whitney test, and from challenged control group ($P < 0.001$).

There was no evidence that repeated injections of oncospheres could stimulate any host-mediated destruction or limitation of these cysts. However, in none of the lambs receiving repeated injections were cysts observed at other than the first injection site.

Lambs were partially resistant to a challenge infection with *E. granulosus* eggs 14 days after a single injection of oncospheres ($P < 0.001$) and 96.7% of the challenge infection was not able to establish (see Table I). After two or more injections, all lambs exhibited a high degree of resistance. One lamb in Group 3 had three dead cysts in the liver and two dead cysts in the lung, and one lamb in Group 4 had one live cyst in a lung.

The cyst-killing ability of sera collected at various times from the primary injection onwards is shown in Table II. In no case did the sera collected after one exposure to antigen, kill all the cysts, but after two exposures, in most cases all cysts were killed. The ability to kill all cysts in vitro appeared to be retained for at least 14 days after the second exposure

to *E. granulosus* oncospheres but then declined to a lower level, where only 55 to 83% of cysts were prevented from developing.

DISCUSSION

The five cysts found in the liver and lung of one sheep in Group 3, and the one cyst in the lung of a sheep in Group 4 could possibly have translocated from the primary injection site. If this did occur it was obviously an infrequent occurrence, as no cysts were found in the liver and lungs of the other 18 sheep in Groups 2 to 5.

The absence of cysts at secondary and later injection sites, the partial resistance to oral infection 14 days after injection, and the death of developing cysts in vitro within 7 days of culture in certain sera, indicated that at least some of the antigens responsible for resistance to establishment of *E. granulosus* cysts are elaborated during the first 14 days of cyst development. The immunizing antigens may actually be in oncospheres, because Xylinas

TABLE II. Mean percentage* of *Echinococcus granulosus* cysts developing after 7 days in vitro in serum collected at the time of each injection, at the time of oral challenge, and at intervals after challenge.

Treatment groups	Days before challenge					Oral challenge 0	Days after challenge		
	-70	-56	-42	-28	-14		+14	+56	+168
Challenged control						95	35	55	71
Group 1					97	29	0	32	29
Group 2				100	46	0	0	21	45
Group 3			99	42	0	0	0	11	41
Group 4		96	47	1	0	0	0	16	27
Group 5	100	32	2	1	0	0	1	20	17
Unchallenged control	100	100	100	100	100	100	100	100	100

* Each percentage figure is a mean of the number of cysts developing in the sera of five sheep, compared to the number of cysts developing in cultures containing serum from unchallenged control sheep.

et al. (1976) were able to immunize mice against infection with *E. granulosus* eggs by repeated, intraperitoneal injection of antigen prepared from *E. granulosus* eggs.

Although the oncosphere or early developing cyst appear to be responsible for the induction of resistance to the establishment of infection, from the present results it is not apparent whether a second exposure to antigen is the important factor, or whether the time taken to mount a completely effective immune response is slightly more than 14 days. Probably the second exposure to antigen is the dominant factor, in view of the incomplete resistance recorded here 14 days after a single subcutaneous injection, and the results obtained by Sweatman et al. (1963) and Gemmell (1966). The observations on in vitro cyst death (Table II) support the contention that a second exposure to antigen is required. Serum from sheep, 14, 56, or 168 days after receiving a single oral infection was not able to kill all developing cysts. In contrast, serum collected 14 days after oral challenge of sheep receiving one or more subcutaneous injections, killed virtually all cysts.

It is not apparent from this experiment as to whether protection will decrease progressively with time. However, the data in Table II for in vitro development of cysts in serum collected 2 wk, 2 or 6 mo after infection support this possibility. Even with animals that were totally resistant to oral challenge following two or more injections, the cyst-killing ability of these sera appeared to decrease within 2 mo of the last exposure to antigen.

All the cysts examined in this experiment 6 mo after infection, whether in subcutaneous sites or in the liver and lung, contained fluid and appeared viable except for those from one lamb in Group 3. There was no consistent difference in size of cyst, or thickness of cyst wall, between the liver and lung cysts of controls and lambs injected once. Thus, there was no indication that an enhanced death rate of established cysts would occur in lambs receiving a single injection, as suggested by Gemmell (1966), or even following repeated injections. Nevertheless, in lambs receiving two or more injections, no cysts were observed at the site of injections subsequent to the first. We have shown previously that the site of a primary injection does not influence the ability of the cysts to grow at that site.

This, therefore, contrasts markedly with an oral challenge following a primary injection, which did result in a few cysts. Presumably the host response to a second injection contained within a defined subcutaneous site was more effective than that to an oral infection where cysts are widely dispersed through liver and lungs.

The results of this experiment thus indicate that, using this technique, it does not appear possible to stimulate a cure for echinococcosis. However, for prophylactic purposes it is possible to stimulate in sheep a very high degree of resistance to oral infection. This resistance, following two or more exposures to antigen, is significantly greater than the partial resistance resulting from a single exposure. Further work should be conducted with a non-living vaccine prepared either from oncospheres or from metabolic and secretory products of cysts developing for up to 14 days in vitro.

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