Immunology, 1962, 5, 496.

Natural and Acquired Immunity Factors Interfering with Development During the Rapid Growth Phase of *Echinococcus granulosus* in Dogs

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(*Received 10th January* 1962)

Summary. Twenty-five dogs were used in a vaccination trial against infections of *Echinococcus granulosus*. Nine of these acted as controls and sixteen were vaccinated with freeze-dried preparations from either tapeworm tissue or scolices of *E. granulosus*. All were challenged with an aliquot of living scolices and examined at periods up to 49 days after challenge.

The results indicate that 0.1 g. of either crude antigen complex did not prevent some worms developing from a subsequent challenge with about 50,000 scolices. However, the number of segments and lengths of the subterminal and terminal segments of *E. granulosus* were almost always less than those found in controls. A phase of rapid growth of the terminal and subterminal segments was observed in control dogs. This was absent in practically all worms in vaccinated dogs and appears to be essential for egg production.

INTRODUCTION

Although Turner, Berbarian and Dennis first published the results of some vaccination trials in 1933, it is only in recent years that additional evidence has been recorded by Matov and Vasilev (1955) and by Forsek and Rukavina (1959) indicating that immunization of dogs may effect subsequent *Echinococcal* infections.

The limited objectives of the trial reported in this paper were to determine whether the injection of *Echinococcal* antigens would prevent the establishment of a subsequent challenge infestation or reduce the worm burden or interfere with the rate of segmentation, growth and reproductive capacity of *Echinococcus granulosus* in dogs.

MATERIALS AND METHODS

DESIGN. Twenty-five dogs were divided into three groups (see Table 1). Nine dogs (group 1) acted as controls. Five of these were young puppies (group 1a) and four were mature or old dogs (group 1b).

Sixteen dogs were vaccinated in this trial. Eleven dogs (group 2) were vaccinated with freeze-dried scolices. Of these, three were young dogs (group 2a) injected with scolices administered as one injection with adjuvant and five were mature or old dogs (group 2b) injected in a similar manner. The remaining three dogs vaccinated with scolices were young puppies (group 2c); each of these received freeze-dried scolices as five injections in water.

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Five young dogs (group 3) were vaccinated with freeze-dried tapeworms either as a single injection with adjuvant (group 3a) or as five injections in water (group 3b).

PREPARATION OF ANTIGEN. Scolices from hydatid cysts collected from sheep were freeze-dried, ground to a fine powder and stored at −5°. Included with the scolices were parts of the germinal and laminated membranes.

Echinococcal tapeworms were collected from the washed intestinal contents of dogs experimentally infected with material from sheep. These worms were freeze-dried, powdered and stored in the same manner as the scolex antigen.

ADJUVANT VACCINE. The adjuvant was a water-in-oil emulsion consisting of 12 parts of a saline suspension of ground-up freeze-dried scolices or tapeworms, 10 parts of liquid paraffin (Shell Ondina 17) and 2 parts of anhydrous lanoline mixed together in a blender.

DOSE RATE.—Dried powdered scolices or tapeworm tissue were injected either as 0.1 g. suspended in 10 ml. of adjuvant and given as a single injection to dogs in groups 2a, 2b and 3b, or given as five injections of 0.02 g. in 2 ml. of water at 5-day intervals to dogs in groups 2c and 3a (see also Table 1). Methiolate, 1/10,000, was added to the vaccine.

All injections were given intramuscularly.

TABLE 1

METHODS AND MATERIALS USED IN VACCINATION TRIAL

Antigen group		*Method of preparation of antigen dose*	*Dog identity*	*Approximate age (years)*	*Brief description*	*Interval (days) between Vaccination and challenge*	*Challenge and autopsy*
Group 1a	Control	—	V.32	½	Small mongrel	—	21
,,	,,	—	V.23	½	Small terrier ×	—	28
,,	,,	—	V.33	½	,,	—	35
,,	,,	—	V.34	½	,,	—	42
,,	,,	—	V.24	½	,,	—	49
Group 1b	,,	—	V.30	Mature	Large Labrador ×	—	28
,,	,,	—	V.27	Mature	Large Labrador	—	35
,,	,,	—	V.28	Mature	Red Setter	—	42
,,	,,	—	V.25	Mature	,,	—	49
Group 2a	Scolex	0.1 g. in 10 ml. adjuvant	V.17	½	Small terrier	41	35
,,	,,	0.1 g. in 10 ml. adjuvant	V.19	½	,,	41	42
,,	,,	0.1 g. in 10 ml. adjuvant	V. 7	½	,,	41	49
Group 2b	,,	0.1 g. in 10 ml. adjuvant	V. 4	Mature	Large terrier	56	28
,,	,,	0.1 g. in 10 ml. adjuvant	V. 5	Mature	Terrier	41	28
,,	,,	0.1 g. in 10 ml. adjuvant	V. 2	Mature	Bull terrier	56	35
,,	,,	0.1 g. in 10 ml. adjuvant	V. 3	Mature	Mongrel	56	42
,,	,,	0.1 g. in 10 ml. adjuvant	V. 1	Mature	Labrador ×	56	49
Group 2c	,,	0.02 g. in 2 ml. water × 5 at 5-day intervals	V.11	½	Small terrier	41	35
,,	,,	0.02 g. in 2 ml. water × 5 at 5-day intervals	V.13	½	,,	41	42
,,	,,	0.02 g. in 2 ml. water × 5 at 5-day intervals	V.20	½	,,	41	49
Group 3a	Tapeworms	0.02 g. in 2 ml. water × 5 at 5-day intervals	V.15	½	,,	41	14 (died)
,,	,,	0.02 g. in 2 ml. water × 5 at 5-day intervals	V.22	½	,,	41	42
,,	,,	0.02 g. in 2 ml. water × 5 at 5-day intervals	V.14	½	,,	41	49
Group 3b	,,	0.1 g. in 10 ml. adjuvant	V. 9	½	,,	41	42
,,	,,	0.1 g. in 10 ml. adjuvant	V.10	½	,,	41	49

FEEDING AND MANAGEMENT. All animals were maintained on a similar diet of raw beef heart, dog biscuits and water *ad lib.* Dogs were examined for tapeworms and eggs before the start of the experiment and under the conditions of management it is unlikely that infections occurred other than by challenge.

CHALLENGE. Scolices from sheep cysts were thoroughly mixed and an aliquot of 0.25 ml. containing approximately 50,000 scolices were administered *per os* to each of twenty-five dogs within 4 hours of collection. The intervals between vaccination and challenge as well as between challenge and autopsy are shown in Table 1.

CRITERION FOR ASSESSMENT. Four criteria are used in the assessment of the effects of the vaccinations. These are the number of worms found in the intestine at autopsy, the growth and the segmentation rate and the degree of sexual development of the worms in the vaccinated dogs, compared with those of the worms found in the controls killed at the same time.

WORM COUNT. The number of worms was assessed as follows. At post-mortem, the intestine was measured and divided into four equal parts. Each part was thoroughly washed into glass jars. The intestine was then scraped and the scrapings added to the washings. The contents and scrapings from each part of the four sections was then washed by sedimentation until sufficiently clear to allow counting. Worm numbers greater than approximately 100 were counted by sampling.

WORM MEASUREMENT. Worms were relaxed in tap water at room temperature over a period exceeding 12 hours. Both the length and breadth of each segment of each selected worm were measured. Where available twenty worms from each sector were examined.

SELECTION OF WORMS FOR MEASUREMENT AND MORPHOLOGICAL STUDY. As many of the worms were damaged and it could not always be ascertained whether a worm was complete, random selection was not attempted, but the largest worms were selected.

RESULTS

EFFECT OF VACCINATION ON THE WORM BURDEN

An infection was established in all nine control dogs and in fifteen out of the sixteen vaccinated animals.

The average number of worms established in the nine controls was 4120 (variation 13 to 9063), whereas the average in the sixteen vaccinated animals was only 1483 (variation 0 to 4561). The group of vaccinated animals with the lowest average worm burden (220) was that of mature dogs (Table 2).

The average worm burden was lower in young dogs injected with tapeworm antigen (864) than that in dogs injected with scolex antigens (3049).

GROWTH AND SEGMENTATION RATE OF *E. granulosus* IN CONTROL AND VACCINATED DOGS

Control dogs

Fig. 1* shows that there is, in young dogs, a steady growth of each worm. By the

* The worm measurements for this figure were selected as explained in 'Materials and Methods'. Each segment represented in the diagram is the average reading of every segment of up to twenty worms in the first and second sector of the bowel. An additional figure has been included for V.20, because large worms were found in the third sector only. Not all the worms selected in the same dog had the same number of segments. For example, some of the worms measured had only two segments whereas others had three or four. Where this occurred the average was taken as three or four and the segment or segments distal to the scolex has been drawn in the figure as occurring in all the worms.

TABLE 2

VARIATION IN WORM BURDENS BETWEEN CONTROL AND VACCINATED DOGS CHALLENGED WITH *E. granulosus*

				Average worm burden
Controls	Group 1a	5 young dogs		5457
	Group 1b	4 mature dogs		2449
	TOTAL	9 dogs		4120
Scolex vaccine	Group 2a	3 young dogs:	scolex and adjuvant	2924
	Group 2b	5 mature dogs:	scolex and adjuvant	220
	Group 2c	3 young dogs:	scolex without adjuvant	3174
	TOTAL	11 dogs		1764
Tapeworm	Group 3a	3 young dogs:	tapeworm without adjuvant	488
	Group 3b	2 young dogs:	tapeworm and adjuvant	1429
	TOTAL	5 dogs		864
ALL VACCINES TOTAL		16 dogs		1483

21st day, the majority of worms had developed two segments. Between the 28th and 35th days two further segments had been added by many worms.

Table 3 shows the variations in the total lengths of *E. granulosus* as well as in the lengths of the terminal and subterminal segments observed in young and mature dogs at different intervals of time up to 49 days after challenge. Growth and segmentation rates were not uniform for all worms in the same sector or between sectors of the bowel in the same dog.

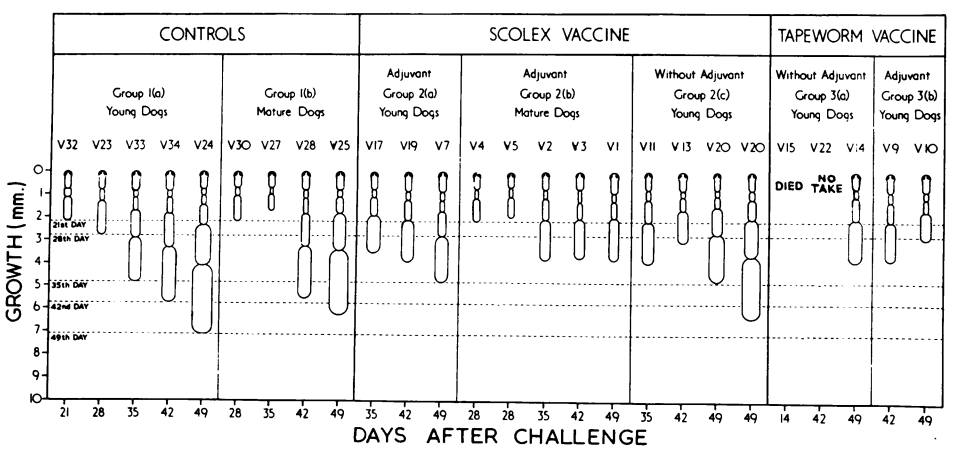

FIG. 1. Segmentation rate and average growth rate of segments of *E. granulosus* in control and vaccinated dogs (scale ×20).

The most advanced worms in young dogs exceeded 2.5 mm. at 21 days; 3.5 mm. at 28 days; 5.5 mm. at 35 days; 7 mm. at 42 days and 8 mm. at 49 days. At 49 days the total lengths varied between 4 mm. and 8 mm. and the number of segments varied from three to five. Although the third and fourth segments were produced between the 28th and 35th days, there was little increase in the length of them up to the 49th day. It is mainly, therefore, the growth of the terminal and subterminal segments that account for the increase in size of *E. granulosus* up to the 49th day. The lengths of the terminal segments measured

at 21 days varied from 0.8 mm. to 1.4 mm.; at 28 days from 0.9 mm. to 2 mm.; at 35 days from 1.3 mm. to 2.3 mm.; at 42 days from 2.1 mm. to 3.3 mm.; and at 49 days from 1.9 mm. to 3.5 mm. The terminal and subterminal segments nearly all doubled their lengths and began to develop sexually between the 28th and 42nd days. On the 49th day, the subterminal segments were similar in size to those of the terminal segments at 35 days. There appeared to be stages of slow growth followed by periods of intense growth with intervals of up to 14 days between each segment in the strobila.

There was a tendency for average lengths of *E. granulosus* in the last two bowel sectors to be slightly less than those in the first two sectors.

Growth of individual worms in mature dogs may be as great as those in young dogs, but the average lengths of the terminal and subterminal segments were always less than the average found in young dogs killed at the same time.

TABLE 3

VARIATIONS IN THE LENGTHS (MM.) OF THE SUBTERMINAL, TERMINAL SEGMENTS AND OF THE TOTAL LENGTHS OF *E. granulosus* IN CONTROL DOGS

Age of infection (days)	*Group 1a Young dogs*				*Group 1b Mature dogs*			
	Dog identity and no. of worms found		*Lengths Max.*	*Min.*	*Av.*	*Dog identity and no. of worms found*	*Lengths Max.*	*Min.*	*Av.*
21	V.32 4211	ST	0.6	0.3	0.4	—	—		
		T	1.4	0.8	1.1	—	—		
		W	2.9	1.6	2.2	—	—		
28	V.23 2851	ST	0.9	0.3	0.5	V.30 13	0.5	0.3	0.3
		T	2.0	0.9	1.3		1.3	0.8	1.0
		W	3.6	1.6	2.6		2.7	1.7	2.0
35	V.33 4549	ST	1.6	0.5	1.0	V.27 1553	0.6	0.2	0.3
		T	2.3	1.3	1.8		1.1	0.3	0.8
		W	5.6	1.6	3.5		2.6	1.0	1.7
42	V.34 9063	ST	2.0	1.0	1.5	V.28 1313	2.0	0.9	1.3
		T	3.3	2.1	2.4		2.8	1.8	2.1
		W	7.4	3.2	5.0		6.5	3.1	4.8
49	V.24 6610	ST	2.1	1.3	1.7	V.25 6918	1.9	0.6	1.3
		T	3.5	1.9	2.9		3.6	1.3	2.4
		W	8.2	4.3	6.6		7.6	3.0	5.3

ST: subterminal segments; T: terminal segments; W: whole worms including scolex.

Vaccinated dogs

The figure shows that in only one animal (V.20) of the fifteen vaccinated dogs were growth processes of *E. granulosus* almost as advanced as in young control dogs killed at the same time. Although the scolex and two segments distal appear to be similar in size to those in control dogs, the number of segments did not exceed three in any worms in fourteen of these dogs and there was retardation with, in some dogs, complete arrest of growth of all the terminal and subterminal segments between the 35th and 49th days after infection.

TABLE 4

VARIATIONS IN LENGTHS (MM.) OF THE SUBTERMINAL, TERMINAL SEGMENTS AND TOTAL LENGTHS OF *E. granulosus* IN VACCINATED DOGS

Age of infection (days)	*Scolex vaccine*								*Tapeworm vaccine*			
	Group 2a Young dogs		*Group 2b Mature dogs*		*Group 2c Young dogs*		*Group 3a Young dogs*		*Group 3b Young dogs*			
	Dog identity and No. of worms found	*Lengths Max. Min. Av.*	*Dog identity and No. of worms found*	*Lengths Max. Min. Av.*	*Dog identity and No. of worms found*	*Lengths Max. Min. Av.*	*Dog identity and No. of worms found*	*Lengths Max. Min. Av.*	*Dog identity and No. of worms found*	*Lengths Max. Min. Av.*		
28	—	ST —	V.4 281	0.9 0.3 0.4	—	—	—	—	—	—	—	—
		T —		1.6 0.7 1.0	—							
		W —		2.6 1.5 2.2	—							
28	—	ST —	V.5 125	0.5 0.1 0.4	—	—	—	—	—	—	—	—
		T —		1.5 0.5 0.9	—							
		W —		2.6 1.0 1.4	—							
35	V.17 2000	ST 1.0 0.4 0.8	V.2 60	1.4 0.3 0.9	V.11 4508	1.3 0.5 0.8	—	—	—	—	—	—
		T 1.8 0.8 1.4		2.0 1.1 1.7		2.1 1.3 1.7						
		W 4.0 2.1 3.4		4.4 1.4 2.9		4.7 1.9 3.3						
42	V.19 2310	ST 1.6 0.5 0.8	V.3 151	1.0 0.4 0.8	V.13 454	1.1 0.4 0.6	V.22 0	No take	V.9 32	1.3 0.5 0.9		
		T 2.8 1.0 1.5		2.6 1.0 1.7		2.0 1.0 1.4				2.3 1.3 1.8		
		W 6.1 1.9 3.3		4.0 2.2 3.5		4.8 2.6 2.8				4.4 2.7 3.6		
49	V.7 4461	ST 1.8 0.5 1.0	V.1 484	0.8 0.3 0.6	V.20 4561	2.1 0.8 1.4	V.14 723	1.4 0.5 1.0	V.10 2826	1.1 0.5 0.8		
		T 3.3 1.3 1.9		1.8 0.8 1.2		3.6 1.3 2.6		2.8 1.0 1.9		2.1 1.3 1.7		
		W 6.9 2.7 4.1		4.3 3.0 3.8		8.3 3.1 5.1		5.3 2.3 3.9		3.6 2.0 2.7		

ST: subterminal segments; T: terminal segments; W: whole worms including scolex.

The average lengths (Table 4) of the terminal segments of *E. granulosus* in young dogs (V.19, V.7, V.13 and V.20) vaccinated with scolices, killed on the 42nd and 49th days was similar to the average length of the terminal segment found in the young control dog (V.33) killed on the 35th day.

However, a few terminal and subterminal segments of *E. granulosus* in these young dogs vaccinated with scolices underwent a period of rapid growth as maximum lengths (2.0 mm. to 3.6 mm.) of the terminal segments were similar to those found in control dogs (V.34 and V.24) killed at the same time. This occurred mainly in V.20 and was almost completely confined to worms in the third sector of the bowel. The finding of well-developed worms almost exclusively in the third sector of V.20 appears to be unusual.

In mature dogs (V.3 and V.1) vaccinated with scolices and young dogs (V.14, V.19 and V.10) vaccinated with tapeworm antigen, all killed on or after the 42nd day, the average lengths of the terminal segments varied between 1.2 mm. and 1.9 mm. These lengths were also similar to those found in controls killed on the 35th day, but maximum lengths of the subterminal and terminal segments in all dogs in these groups (2b, 3a and b) were considerably less than those found in the controls (V.34 and V.24) killed at the same time.

Whilst a few subterminal and terminal segments of *E. granulosus* in dogs in these groups lengthened slowly, none, unlike those observed in young dogs vaccinated with scolices, went through a period of rapid growth between the 28th and 49th days.

DISCUSSION

Turner *et al.* (1933), Matov and Vasilev (1955), Forsek and Rukavina (1959), showed that a marked reduction in worm burden occurred in most of their dogs vaccinated with hydatid fluid, membranes or scolices and in some instances no tapeworms developed.

As the average worm burdens and the lengths of the terminal and subterminal segments of almost all worms in vaccinated dogs were less than that found in control dogs of equivalent age, it is not unreasonable to infer that an antibody-antigen reaction was responsible for the results also obtained in this trial.

The rapid growth of the subterminal and terminal segments of *E. granulosus* between the 35th and 49th days in control dogs suggests a period of 'stress' for the parasite with an increased metabolism. As almost all the subterminal and terminal segments in vaccinated dogs failed to undergo a period of rapid growth between the 35th and 49th days, it appears that the more important antigen-antibody reactions in this trial were associated with inhibitions to growth.

The fact that some worms overcame inhibitions to growth in young dogs vaccinated with material from scolices, but none did so in young animals vaccinated with material from adult tapeworms, and that the average worm burdens were smaller in the latter, suggests that there may be quantitative or qualitative differences, in the antigens, between the two crude materials used in this trial.

The observation that growth of segments of *E. granulosus* may be slightly retarded in mature dogs, as compared with young dogs, suggests an age resistance factor. This may have only a slight effect (cf. V.24 with V.25) on growth or be marked (cf. V.33 with V.27). If an age resistance factor does occur either as a natural phenomenon or acquired by experience, as suggested in the field (Gemmell, 1959), it might account for differences in the effectiveness of the scolex vaccine in retarding growth of all worms and decreasing

worm burdens in mature dogs as compared with young dogs injected with the same material.

No developing eggs were seen in terminal segments of *E. granulosus* which failed to undergo rapid development between the 35th and 49th days and, as Matov and Vasilev (1955) have shown that worms in their vaccinated dogs did not reach sexual maturity after challenge even up to 88 days, it appears that, unless segments can overcome inhibitions to growth, fertilization may not occur or at least may be delayed for a considerable period.

ACKNOWLEDGMENTS

This work was undertaken whilst the author held the George Aitken Pastoral Research Scholarship tenable in the Department of Veterinary Pathology and Bacteriology, University of Sydney, Australia. The figure was prepared by the Illustrations Department of the Medical School, Dunedin, New Zealand.

Mr. R. V. S. Bain, Senior Lecturer in the Department of Veterinary Pathology and Bacteriology, University of Sydney, prepared the adjuvant used in this trial.

Dr. I. W. Parnell of this unit, Professor H. R. Carne, Department of Veterinary Pathology and Bacteriology, University of Sydney, and Mr. H. McL. Gordon, Senior Principal Research Officer, Commonwealth Scientific and Industrial Research Organization, McMaster Laboratory, Sydney, gave valuable advice and help during the trial and in the preparation of this manuscript.

To all these I wish to record my appreciation.

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