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Experimental infections of rabbits and guineapigs with *Trichostrongylus axei* (Cobbold, 1879) Railliet and Henry, 1909.

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Summary

Rabbits and guineapigs were inoculated with different doses of infective larvae of *Trichostrongylus axei per os* and autopsied on 7th, 14th and 28th days after infection. The total number of worms recovered increased with the dose of the larvae administered but the percentage establishment of the worms decreased with increase in dose of the infective larvae. The worms were localised in stomach and small intestine ; the majority being recovered from the stomach. The infected rabbits and guineapigs either lost weight or gained less weight than those of non-infected animals. The worms recovered on 7th and 14th days were immature while those on 28th day were adult. The size of the worms was smaller than those from the normal hosts.

Rabbit was found to be a suitable host for experimental studies on *Trichostrongylus axei*. Guineapig was considered to be a relatively less receptive host for this parasite.

Résumé

Infections expérimentales de lapins et de cobayes par* Trichostrongylus axei *(Cobbold, 1879), Railliet et Henry, 1909.

Les différentes doses de larves infestantes de *Trichostrongylus axei* ont été administrées *per os* aux lapins et aux cobayes. Les animaux étaient autopsiés les 7e, 14e et 28e jours après l'infection. Le nombre total de vers retrouvés augmentait avec la dose des larves administrées mais le pourcentage des vers établis diminuait avec l'augmentation de la dose

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des larves. Les vers étaient localisés dans l'estomac et dans l'intestin grêle, la majorité de ceux-ci a été retrouvée dans l'estomac. Les lapins et les cobayes infectés ont perdu ou ont gagné un poids inférieur à celui des animaux non-infestés. Les vers recueillis les 7e et 14e jours étaient immatures tandis que ceux du 28e jour étaient adultes. La taille des vers était plus petite que celle des vers de l'hôte normal.

On a constaté que le lapin est un hôte convenable pour l'étude expérimentale de *Trichostrongylus axei*. Le cobaye est considéré comme un hôte relativement peu réceptif à ce parasite.

Trichostrongylus axei is recognised as an important pathogen of ruminants and horses in many parts of the world. Its natural infection in a wild rabbit in New Zealand was reported by Bull (1953). Drudge *et al.* (1955) for the first time successfully infected rabbits with equine, bovine and ovine strains and guineapigs with the equine and bovine strains of *T. axei*. Leland and Drudge (1957) made studies on quantitative aspects of experimental infections in rabbits. Thereafter the rabbits have been used in some more studies (Rohrbacher, 1960 ; Ciordia *et al.*, 1966 ; Sinha, 1967). Cauthen (1958) reported that rabbits are not satisfactory animals for tests of anthelmintics to be used against *T. axei* in ruminants.

High costs of domestic animals is a limiting factor in their use for experimental purposes in the study of nematodes like *T. axei*. Technically and economically it is more easier and advantageous to maintain and study a pathogen of large domestic animals in small laboratory animals. This prompted us to take up this study. The value of guineapigs as laboratory host of this parasite was tested in detail. At the same time some experiments on rabbits were also made to verify the previous reports.

Materials and methods

Young rabbits and guineapigs bred and reared in the laboratory were used. They were kept either individually or in groups in wire mesh cages. Commercial pelleted food and water was supplied daily *ad. lib.* Both male and female animals were used.

T. axei was maintained as a pure infection by administering infective larvae to a worm free calf. This calf served as the source of infective larvae. The faeces obtained from the rectum were cultured at 25 °C for 2 weeks and the infective larvae recovered by baermannization. All the animals were dosed with the larvae from the same suspension. Only freshly harvested larvae were used.

The required number of infective larvae suspended in water were administered *per os*, using a syringe fitted with a 8 cm. length of plastic tubing for guineapigs and a more longer tube for rabbits. After administration of the larval dose the syringe and tubing were washed with 0.5 ml. of water and this 'wash' was also administered. The whole larval dose was concentrated in not more than 2 ml. of water for guineapigs and 5 ml. for rabbits.

The animals were killed by ether or chloroform vapour. Immediately afterwards, stomach and small intestine were seperated. The stomach was opened, its contents

removed and then placed in pepsin-hydrochloric acid digestion fluid (Herlich, 1956). The intestine was cut into small pieces and placed in another container filled with the same digestion fluid. Digestion was allowed to proceed over night at 37 °C. The stomach contents and digests of stomach and intestine were poured onto a 100 and then onto a 200 mesh sieve to allow small particles and fluid to pass through. The material retained on the sieve was washed off carefully by a jet of water. The search was made with the aid of a dissecting microscope for the recovery of the worms. If immediate counting was not possible, whole material was preserved in 10 % formaline.

Sixteen rabbits were used in the experiments. Two larval doses were utilised : 4,000 and 8,000. For 4,000 larval dose level, 1 animal was autopsied on day 7, 2 animals were autopsied on day 7, 2 on day 14 and 3 on day 28 post infection. Three animals were autopsied on day 7.2 on day 14 and 3 on day 28 post infection. Three rabbits were kept as controls. All the rabbits were weighed at the beginning of the experiments. Those distined to be sacrificed on day 28 post infection and control group were weighed weekly to follow the live body weight changes.

Seventy guineapigs, divided into 10 equal groups, were used. Each group had animals of both sexes. Three levels of larval doses were utilised : 1,000, 4,000 and 8,000. Three groups of animals were infected with each dose level. Thus there were 9 experimental and 1 control group. One group from each dose level was autopsied on days 7, 14 and 28 days post infection. To follow the changes in the body weight, the guineapigs destined to be autopsied on 28th day and control group were weighed weekly. All other animals were weighed only at the beginning of the experiment.

Results

Rabbits.

Data pertaining to sex, initial body weight, larval doses, days of autopsy and the worms recorvered on *post mortem* are presented in table I.

Number of worms increased with the dose of the larvae. But the percentage establishment did not increase even when the larval dose was doubled. Analysis of variance showed that there was no significant difference between the percentage worm recoveries from rabbits of different groups. Although many of the females harboured lesser number of worms than the males, the individual differences were so high that it may be assumed that there were no real differences between the susceptibility of male and female rabbits.

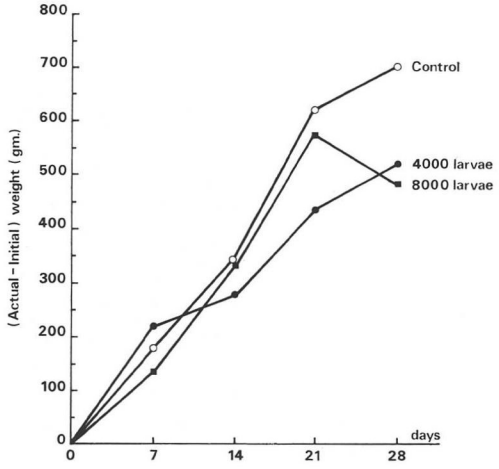
The worms were distributed in stomach as well as in the small intestine. Although in some rabbits a significantly good number of worms were found in the small intestine the majority of them were located in the stomach.

The mean body weight gains of different groups of rabbits are presented in fig. 1. Animals in all groups put weight. Statistical analysis showed that a trend was

Table I. — Recovery of *Trichostrongylus axei* from rabbits.

Rabbit N°	Sex	Weight (gm)	Dose of larvae	Days from infection to autopsy	Worms recovered			Percent of larvae administered
					Stomach	Small intestine	Total	
1	F	1750	4.000	7	890	120	1010	25,3
2	M	2580	4.000	14	700	200	900	22,5
3	M	1305	4.000	14	480	150	630	15,8
4	M	2400	4.000	28	1050	150	1200	30,0
5	F	1420	4.000	28	890	50	940	23,5
6	M	1800	4.000	28	1000	45	1045	26,1
7	F	1420	8.000	7	1800	200	2000	25,0
8	M	1440	8.000	7	1550	250	1800	22,5
9	F	1765	8.000	14	1100	500	1600	20,0
10	M	1910	8.000	14	1500	200	1700	21,3
11	F	1820	8.000	28	1080	325	1405	17,6
12	M	2055	8.000	28	1300	785	2085	26,1
13	F	1755	8.000	28	990	310	1300	16,3

M = Male.
F = Female.



observed in the weight gain of animals according to the dose of the larvae. The increase in weight was continuous in control group and in the group of 4,000 larval dose, whereas in the group of the 8,000 larval dose there was a drop in weight gain in the 4th week. This corresponds with the time when the worms become mature. It seems, therefore, that a minimum of 8,000 larvae are necessory to have an appreciable effect on the body weight gain of rabbits.

FIG. 1. — Mean body weight changes of 3 groupes of rabbits

Guineapigs.

Data pertaining to sex, body weight, larval dose, days of autopsy and worm recoveries on *post mortem* examination are given in tables II, III, IV.

Table II. — Recovery of *Trichostrongylus axei* from guineapigs infected with 1000 larvae.

Guineapig No.	Sex	Weight (gm)	Days from infection to autopsy	Worms recovered			Percent of larvae administered
				Stomach	Small intestine	Total	
1	M	545	Died	—	—	—	—
2	M	700	7	140	10	150	15.0
3	M	300	7	90	0	90	9.0
4	F	450	7	130	15	145	14.5
5	F	440	7	45	0	45	4.5
6	F	400	7	0	0	0	0.0
7	F	325	7	10	0	10	1.0
Average		451		69	4	73	7.3
8	F	355	Died	—	—	—	—
9	F	365	14	45	25	70	7.0
10	F	365	14	100	10	110	11.0
11	F	360	Died	—	—	—	—
12	M	220	14	90	40	130	13.0
13	M	260	14	15	0	15	1.5
14	M	305	14	0	0	0	0.0
Average		319		50	15	65	6.5
15	F	555	28	0	0	0	0.0
16	F	385	28	40	50	90	9.0
17	M	260	28	45	35	80	8.0
18	M	340	28	100	25	125	12.5
19	M	405	28	15	5	20	2.5
20	M	320	28	35	15	50	5.0
21	F	430	Died	—	—	—	—
Average		385		39	22	61	6.1

M = Male

F = Female

Table III. — Recovery of *Trichostrongylus axei* from guinea pigs infected with 4000 larvae.

Guineapig No.	Sex	Weight (gm)	Days form infection to autopsy	Worms recovered			Percent of larvae adminis-tered
				Stomach	Small intestine	Total	
22	F	360	7	405	0	405	10.1
23	F	640	7	307	13	320	8.0
24	F	385	7	115	15	130	3.3
25	M	375	7	450	125	575	14.4
26	M	475	7	535	125	660	16.5
27	M	370	Died	—	—	—	—
28	M	280	Died	—	—	—	—
Average		412		362	56	418	10.5
29	M	515	14	500	0	500	12.5
30	M	550	Died	—	—	—	—
31	M	410	14	315	112	427	10.7
32	F	385	14	138	22	160	4.0
33	F	330	14	125	10	135	3.4
34	F	320	14	0	0	0	0.0
35	F	240	Died	—	—	—	—
Average		393		216	29	245	6.1
36	F	360	28	130	150	280	7.0
37	F	190	28	435	120	555	14.0
38	M	380	28	0	0	0	0.0
39	M	610	28	15	5	20	0.5
40	M	395	28	105	15	120	3.0
41	M	340	28	285	65	350	9.0
42	F	315	Died	—	—	—	—
Average		367		162	59	221	5.0

M = Male
F = Female

There were great variations in the number of worms recovered from individuals of the same group as well as between the groups. However in general the number of worms increased with the dose of the larvae, but the percentage of worms established decreased with increase in the larval dose. For example, the percentage establishment in the 3 groups of animals autopsied on 28 days after infection was 6.1, 5 and 4.2 for the larval doses of 1,000, 4,000 and 8,000 respectively. Majority

Table IV. — Recovery of *Trichostrongylus axei* from guinea pigs infected with 8000 larvae.

Guineapig No.	Sex	Weight (gm)	Days from infection to autopsy	Worms recovered			Percent of larvae administered
				Stomach	Small intestine	Total	
43	M	430	7	240	80	320	4.0
44	M	330	7	1050	15	1065	13.3
45	M	395	7	0	0	0	0.0
46	F	295	7	515	125	640	8.0
47	F	350	7	135	9	144	1.8
48	F	260	Died	—	—	—	—
49	F	280	7	235	120	355	4.4
Average		334		363	58	421	5.3
50	M	270	14	115	25	140	1.8
51	M	450	14	218	17	235	2.9
52	M	500	14	910	15	925	11.6
53	F	280	14	0	0	0	0.0
54	F	255	14	136	234	370	4.6
55	F	525	14	495	65	560	7.0
56	F	230	14	317	23	340	4.3
Average		359		313	54	367	4.6
57	F	235	28	105	15	120	1.5
58	F	190	28	145	25	170	2.1
59	M	620	28	240	45	285	3.6
60	M	555	28	617	103	720	9.0
61	M	430	28	39	29	68	0.9
62	F	290	Died	—	—	—	—
63	M	370	Died	—	—	—	—
Average		384		289	43	332	4.2

M = Male
F = Female

of the worms were found in the stomach in all the groups. A comparison of the number of worms recovered on different days after infection indicates that as the period of infection advanced, the worm recoveries decreased. It seems that with advance in the period of infection some worms were thrown off. No difference was observed in the susceptibility of male and female guineapigs. There were deaths in all the groups of animals, but these were due to causes other than parasitic.

The mean body weight changes of different groups of guineapigs are shown in fig. 2. The control group had gained 64 gm. of weight at the end of 4 weeks whereas those dosed with 1,000 and 4,000 larvae had gained only 7.5 and 27 gm. respectively. The animals dosed with 8,000 larvae lost 62 gm. of weight during the same period. There were no differences in the body weight changes between the male and female guineapigs. The statistical analysis of variance in the body weight between the different doses was highly significant ($F = 5.25$, $P < 1\ \%$). The

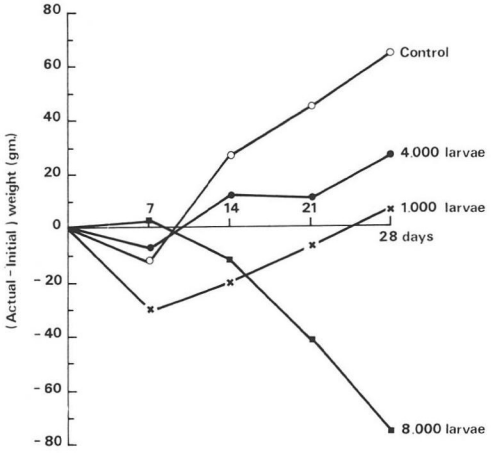


FIG. 2. — Mean body weight changes of 4 groupes of guineapigs

comparison of the averages of different lots by 'Duncan Multiple Range Test' revealed a significant difference in the weight gain (— 62 gm.) of 8,000 larval dose group on one side and the other larval doses on the other side.

The worms recovered on 7th and 14th days after infection were all immature while those recovered on 28th day were mature. The size of the worms from these experimental hosts were comparitively smaller than those from natural hosts of *T. axei*. The worms from guineapigs were significantly smaller than those from rabbits.

Discussion

Several studies with *T. axei* have shown that there is an inverse relationship between the larval dose and the number of worms recovered (Doran, 1955 ; Kates and Turner, 1960 ; Ross *et al.*, 1968). Leland *et al.* (1959) observed great variations in worm establishment (6 to 59 %) of this parasite after its passage in various hosts.

In experimental infections in small laboratory animals the recorded worm recoveries are : 6 to 31 % (Leland and Drudge, 1957), 13.2 to 26.5 % (Sinha, 1967), 1 to 54 % (Ross, 1970) from rabbits ; average 10.5 % from African multimammate mouse, *Rattus natalensis*, (Leland, 1968) ; average 46.3 % (Leland, 1963) and 19.4 % (Kates and Thompson, 1968) from Mongolian gerbils and 0 % from white rats (Kates and Thompson, 1968). The worm recoveries in present experiments from rabbits (15.8 to 30 %) were in the same range as reported by other workers. The establishment of worms in guineapigs (0 to 15 %) was much lower than those in rabbits, gerbils and *Rattus natalensis*. Moreover the individual variations in guineapigs were more marked than in other experimental hosts.

In rabbits, worm recoveries on 7th, 14th and 28th days after infection were identical whereas in guineapigs there were apparent differences in worm recoveries of 28 days and 7 and 14 days after infection. It seems that in guineapigs the worms are thrown off in later period of infection which may be similar to the self cure phenomenon reported for *T. retortaeformis* in rabbits by Michel (1952) and by several other workers in other parasites.

Dunsmore (1966) working with *T. retortaeformis* and *Graphidium stigosum* observed that female rabbits carried more worms than the male ones. This he considered to be an effect of sex hormones. Kates and Thompson (1968) found no significant difference by sex in the susceptibility of white rats and Mongolian gerbils to infection with *T. axei* and *T. colubriformis*. Neither in rabbits nor in guineapigs any difference by sex was found in worm recoveries in the present experiments.

The size of *T. axei* from rabbits and guineapigs was smaller than those from natural hosts. This finding is comparable with shorter lengths of worms from other experimental laboratory animals (Leland, 1963, 1968 ; Ross, 1970).

Sturrock (1963) observed that the body weight change is a good measure of parasitic infection in guineapigs with *T. colubriformis*. In present work the infected rabbits gained relatively less weight than the control ones. The difference in body weight gains in guineapigs infected with 8,000 larvae and other groups was highly significant and it seems that the body weight change in guineapigs may be a good indicator of *T. axei* infections at higher dose levels.

Leland and Drudge (1957) found only few *T. axei* in intestine of some of his experimental rabbits whereas Sinha (1967) recovered relatively high percentages of them from intestine. Since in our experiments both in rabbits and guineapigs majority of the worms were found in stomach, we assume that the stomach is a preferred site for *T. axei* in these experimental hosts.

The present experiments indicate that rabbit can be used as an experimental animal for *T. axei* for different types of studies : pathological, immunological, anthelmintic testing etc. Russel *et al.* (1966) suggested the use of *Obeliscoides cuniculi* infections in rabbits as a model for the study of ruminant *trichostrongylidosis*. It is possible that *T. axei* infections in rabbits may be used as a model for the study of various host parasite phenomenon.

Guineapigs do not seem to be a suitable host for *T. axei.* However they can be employed provided following points are born in mind. Adequate number of animals should be used to allow for variations in the susceptibility of guineapigs. This variation may be minimised if animals of similar age, weight, genetic constitution and similar sex are used.

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