SOME FACTORS AFFECTING THE IMMUNE RESPONSE TO HELMINTH INFECTIONS

by

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THE ROLE OF LARVAE AND ADULT WORMS ON THE IMMUNE RESPONSE TO NIPPOSTRONGYLUS BRASILIENSIS

GENERAL SUMMARY

GENERAL SUMMARY

The original objective of this study was to investigate further, the observation of Benitez-Usher and his colleagues (1977) that when an anthelmintic was administered shortly after each of two immunising infections of irradiated Haemonchus contortus larvae lambs failed to express any immunity to subsequent challenge with normal larvae.

Unfortunately this aim was not achieved due to the fact that the H. contortus irradiated larval vaccine failed to confer protection to subsequent challenge. This result was totally unexpected as in all previously published reports a similar regimen of vaccination was highly successful.

Following this finding it was decided to undertake an investigation of the reasons for the failure of the H. contortus larval vaccine (Section 1) and to use the rat/Nippostrongylus brasiliensis model system to study the original objective i.e. the effect of anthelmintics on the immune response (Section 2).

In examining the possible reasons for the failure of the H. contortus vaccine, the following factors were considered to be most likely.

Firstly, a technical error in the preparation of the vaccine: this was discounted due to the fact that on retrospective checking no errors could be detected. In addition, a research scientist in another institute was found to be currently experiencing similar difficulties with the larval vaccine despite previous success.

The second possibility was that the larvae, due to continuous passage under laboratory conditions, had developed an increased radiosensitivity leading to over-attenuation with resultant loss of both infectivity and immunogenicity. This seemed unlikely

since the worm burdens of the vaccinated groups contained significant numbers of sterile female worms derived from the irradiated larvae. Also, no increased radiosensitivity was observed in an irradiation dose titration experiment in which larvae were irradiated at 40 kr and 60 kr in a ^{60}Co source and administered on a single occasion to lambs. The resultant number of sterile female worms found at necropsy of these lambs when compared with the original vaccine studies carried out in the 1960's, did not indicate any change in the radiosensitivity of the larvae.

The third possibility that the Glasgow strain of H. contortus had lost its immunogenicity was then examined. It was found in two experiments that infection with normal larvae failed to confer the anticipated degree of protection against challenge. It seemed therefore that the strain had lost its immunogenicity, although this conclusion must be tempered by the fact that small groups of lambs were used in these experiments.

Subsequent studies were concerned with a comparison of larvae irradiated at 40 kr and 60 kr in either a ^{60}Co source or an X - ray unit. It was found that irradiation at 40 kr from either source completely restored the immunogenicity of the vaccine to that previously reported i.e. around 95%. However, in the final experiment, irradiation at 60 kr also produced a successful result in that the challenge worm burdens were reduced by 76%. Future experiments are necessary to establish the reason for the inconsistency of the 60 kr vaccine but it is suggested that irradiation at 60 kr is perhaps borderline for the retention of immunogenicity: it is also possible that this variation in results might be associated with the physiological state and innate infectivity of various batches of larvae before being subjected to irradiation.

In experiments with a separate laboratory strain of H. contortus, a significant degree of protection could not be obtained with larvae irradiated at 40 kr which suggests that there are important strain differences in immunogenicity in this species.

The possibility that anthelmintics might modulate the immune response to helminth infections was studied using Nippostrongylus brasiliensis in the rat. Two aspects were investigated.

First, that chemical removal of sensitising worm burdens might produce loss of immunity through the absence of sustained antigenic stimulus rather than any specific immunomodulatory effect. This was studied in a series of experiments where levamisole, thiabendazole and fenbendazole were used to truncate sensitising infections of 10 larvae prior to challenge with 3000 larvae. The results showed that with thiabendazole and fenbendazole there was only a short delay in the full expression of the immune response. With levamisole this delay was even less marked.

Secondly, the immunomodulatory role of anthelmintics was examined by their administration prior to a primary larval infection. There was no firm evidence that levamisole or thiabendazole had any modulatory effect on worm establishment. Also no immunomodulatory effect was observed when young adult worms administered by gastric intubation were removed by these anthelmintics a week before adult worm challenge.

Finally, some observations were made on the relative antigenicity of larvae compared with adult worms. It was shown that although adult worms stimulated an effective immune response against larval or adult worm challenge, large numbers were required to produce the same degree of protection as a small number of larvae.

SECTION 1

IMMUNITY TO *HAEMONCHUS CONTORTUS* INFECTION IN SHEEP.

INTRODUCTION

Although anthelmintics are an integral part of the treatment and control of many important helminth diseases of farm animals throughout the world there has for some years been speculation over the possible effect of anthelmintic treatment on the subsequent development of immunity to helminth infection. This aspect of chemotherapy has received particular attention in Haemonchus contortus infection in sheep which, in tropical and sub-tropical areas of the world is a potential cause of severe morbidity and mortality in sheep and goats. Up to the present time, repeated anthelmintic administration is the only successful control measure which can be applied in H. contortus endemic areas and with the development of new anthelmintics of increased efficiency the possibility that regular use of anthelmintics may affect the development of immunity to H. contortus has interested many parasitologists and veterinarians.

In fact, the practical significance of acquired immunity in H. contortus infection is still not fully understood. Broadly speaking, immunity against this parasite may be expressed in three ways. Through immune expulsion of existing adult infections, commonly referred to as the 'self cure phenomenon' a term coined by Stoll (1929); by prolonged inhibition of ingested larvae at the fourth larval stage (L_4) or even by the failure of ingested larvae to establish and develop.

The 'self cure' phenomenon has been regularly reproduced in sheep maintained under experimental conditions by the administration of infective larvae (Stewart, 1953; Dargie and Allonby, 1975) and Stewart concluded that the probable reason was development of an Immediate-type hypersensitivity reaction in the abomasal mucosa, triggered by the ingested larvae, which in some way made

conditions untenable for the existing population of adult worms. While the phenomenon must undoubtedly occur in the field, Allonby and Urquhart (1973) have recently produced evidence to support Gordon's original observation (Gordon, 1948) that the 'self-cure', as a flock phenomenon, was not necessarily associated with reinfection and was apparently dependent on some 'anthelmintic factor' in freshly growing grass.

Inhibition of larval development associated with repeated experimental infection with H. contortus larvae and apparently attributable to the development of immunity has been described by Dineen and his co-workers (Dineen, Donald, Wagland and Offner, 1965). In the field, larval inhibition has also been observed (Connan, 1971, 1975; Waller and Thomas, 1975; Ogunsusi and Eysker, 1979) but has been generally attributed to hypobiosis induced by environmental stimuli on infective larvae rather than to the development of immunity.

The significance of the third type of immune response, the development of resistance to reinfection, is still not clear. Gordon (1948, 1950) observed that grazing ewes in Australia did not appear to develop a significant immunity to reinfection, an observation corroborated in East Africa by Allonby and Urquhart (1975).

However, there is good evidence from experiments in both Britain and Australia (Jarrett, Jennings, McIntyre, Mulligan and Sharp, 1959, 1961; Manton, Peacock, Poynter, Silverman and Terry, 1962; Dineen and Wagland, 1966) that worm free lambs provided they are over seven or eight months of age can develop a highly significant degree of resistance to challenge with H. contortus after immunisation with irradiated or normal larvae. It has been suggested (Lopez and Urquhart, 1967; Urquhart, 1970) that the

apparent disparity between these two sets of observations might be attributable to repeated reinfection of grazing lambs during the first six months of their lives when they are unable to develop any immunity to H. contortus apparently due to immunological immaturity. Infection during this period might permanently damage the full expression of the immune response. However, the situation is still obscure and further work is required.

Reverting to the effect of anthelmintic treatment on the immune response, Dineen and his colleagues (Dineen and Wagland, 1966; Wagland and Dineen, 1967; Donald, Dineen and Adams, 1969) working with H. contortus infection in lambs in Australia concluded that strong resistance was associated with prolonged uninterrupted infection and that anthelmintic treatment was more likely to interfere with, than assist, the development of immunity. In one study however, there was evidence that persisting high worm burdens produced a state of "immunological exhaustion" in that a very high proportion of a challenge infection developed to maturity. In contrast, when these persistent worm burdens were removed by an anthelmintic prior to challenge, immunity was re-established in terms of the proportion of the challenge infection which matured. From the results of their many experiments Dineen and his co-workers concluded that the use of anthelmintics on a flock basis for the general control of H. contortus in sheep should be approached with some caution and advised limiting their use only to flocks, or even to individuals within flocks, showing clinical evidence of haemonchosis. This suggestion, however, is perhaps rather impractical under the conditions of extensive grazing common in endemic areas.

In vaccination studies in this laboratory Benitez-Usher, Armour, Duncan, Urquhart and Gettinby (1977) observed that when immunologically mature lambs were treated with thiabendazole three weeks after each of two doses of irradiated H. contortus larvae

they failed to develop immunity to subsequent challenge while vaccinated and untreated controls were almost completely resistant. On the other hand, in a similar study by Smith and Christie (1979) a single dose of levamisole administered three weeks after the second vaccination (i.e. a week before challenge) did not affect protection against challenge and they suggested that the continued presence of vaccine worms from weeks three to seven of the immunising course was crucial for the development of the protective response. The possibility that levamisole may have had an adjuvant effect was not discussed.

From these contrasting results it was considered important to investigate further the role of anthelmintics in the development or suppression of immunity to H. contortus. This is however, complex since the loss of immunity may be caused, not merely by the removal by anthelmintic action of potentially sensitising worm burdens, but may also be associated in part with direct immunomodulatory effects of certain compounds; for example, levamisole and thiabendazole have been shown to possess immunostimulant and immunosuppressive properties respectively independent of their anthelmintic activities (see Section 2 - Introduction).

The first two experiments reported in Section 1 were designed to provide some preliminary information on the role of anthelmintics on immunity to H. contortus. Unfortunately this objective was not achieved due to the entirely unexpected failure of the vaccination procedure. The original objective was therefore abandoned and the subsequent experiments in this section were concerned with efforts to determine the cause of this failure. Studies on the immunomodulatory role of anthelmintics were however continued in rats infected with Nippostrongylus brasiliensis and these are reported in the second section of this thesis.

SECTION 1

GENERAL METHODS AND MATERIALS

Animals

Pure bred Scottish Blackface sheep were used in all experiments with the exception of Experiments 5 and 6 when Suffolk-Grey face crosses were used.

Rearing of parasite-free animals

Day old lambs were removed from their mothers and bottle fed indoors with milk substitute. Water and hay were provided from the first week and concentrates were offered from the third week onwards. Lambs were generally weaned around six weeks of age.

The animals were housed in concrete pens which were cleaned thoroughly twice weekly and were provided with fresh straw bedding daily.

All lambs were docked and the males castrated when they were about six weeks old.

Until the beginning of each experiment faecal samples from the lambs were regularly examined for the presence of parasite eggs or larvae.

Body weights

Body weights were determined using a small ruminant weighing crate (Avery Scales Ltd., Glasgow, Scotland).

Strains of Haemonchus contortus larvae

Glasgow strain - This was a strain of H. contortus maintained for over 20 years in the Department of Veterinary Parasitology of Glasgow University by repeated passage in Scottish Blackface lambs.

Merck Sharp and Dohme (MSD) strain - A laboratory strain of H. contortus maintained at MSD research laboratories.

Cambridge strain - This was a recently isolated field strain of H. contortus obtained from Dr. R. Connan of Cambridge University.

Anthelmintics

Levamisole hydrochloride 7.5 percent w/v solution (Nemicide - Imperial Chemical Industries Ltd., Cheshire, England) administered subcutaneously at a dosage rate of 7.5 mg/kg body weight.

Thiabendazole 13.3 percent w/v solution (Thibenzole - Merck Sharp and Dohme Ltd., Hertfordshire, England) administered orally at a dosage of 50 mg/kg body weight.

Parasitological Techniques

Culture and harvesting of *H. contortus* infective larvae

Parasite-free lambs were infected with 5,000-10,000 *H. contortus* infective larvae. From the eighteenth day after infection the animals were faecal sampled daily and when a high number of eggs were detected in the faeces the animals were fitted with faecal bags and thereafter total faecal collections made daily.

Three hundred grams of faecal pellets were placed into each of a series of 500 ml honey jars. A moist filter paper was placed inside each lid, the lids were lightly screwed down and the jars were stored at 22°C for 15 days.

Infective larvae were harvested as follows. The jars were filled with luke-warm tap water and left for one hour to allow the larvae to migrate into the water. The fluid from the jars was then pooled and poured through a sieve (60 meshes per inch) which retained the faecal debris. This fluid containing the larvae was then filtered through two layers of eight inch milk filters (Maxa Filters, A. McCaskie, Stirling) by suction, using a Buchner apparatus and vacuum pump. Larvae retained in the milk filter were recovered using a Baermann apparatus. This consisted of a glass funnel closed at the stem with a length of rubber tubing and a clip, and filled with luke-warm tap water; a sieve with an aperture size of 150 microns

was placed touching the surface of the water. The filter with the retained larvae was then placed on top of the sieve so that the motile larvae were allowed to migrate into the water. After 12 hours the larvae were collected from the neck of the funnel.

Larval counting technique

Larval suspensions were mixed thoroughly to prevent clumping and a suitable dilution was made for counting. From this well mixed suspension forty 0.025 ml aliquots were taken and placed on glass slides for counting. The total number of larvae counted was the number of larvae per ml of the diluted suspension.

Required doses of larvae were placed in universal bottles and administered orally to the animals making sure that no larvae remained in the bottle.

For each infection larvae were freshly harvested from recent cultures.

Faecal egg counts

Faecal samples were collected from the rectum and examined by the modified McMaster technique (Gordon and Whitlock, 1939). In this technique three grams of faeces were homogenised in 42 ml of tap water using an MSE homogeniser. The resulting mixture was passed through an Endecott sieve of aperture size 250 microns and 15 ml of the well mixed filtrate transferred to a flat bottomed test tube. After centrifugation at 2000 rpm for two minutes, the supernatent was discarded and the sediment agitated using a whirlimixer (Scientific Industries Ltd.). The tube was then filled to the previous level with saturated salt (NaCl) solution and inverted six times to ensure thorough mixing.

Using a Pasteur pipette sufficient fluid was removed to fill both chambers of a McMaster slide (Hawksley and Sons, London, England). As the eggs rise rapidly the pipetting must be accomplished quickly.

Under a microscope the number of eggs in both chambers of the McMaster slide was counted and multiplied by 50 to give the total number of eggs per gram (epg) of faeces.

Irradiation procedure

γ - irradiation of H. contortus larvae

The irradiation of larvae was carried out in a "Hot Spot" type ^{60}Co irradiation unit (Gamma chamber Mark IV B, Nuclear Engineering Ltd., Reading, England) calibrated periodically by Fricke dosimetry and by a 'red perspex dosimeter'.*

A known concentration of H. contortus larvae was pipetted into perspex test tubes held in a circular perspex rack which was then placed in the central column of the ^{60}Co unit and lowered mechanically into the irradiation chamber.

The length of time for which larvae were exposed to the γ - rays depended on the output of the machine and the irradiation dose required.

X - irradiation of H. contortus larvae

The X - ray machine used was a "Siemens Stabilipan 300" (Erlangen, Germany). At the time of larval irradiation the machine was operated at 180 kV, 20 mA with an external filtration of 0.1 mm Cu and 1.0 mm Al giving radiation of HVL 8 mm Al.

A known concentration of H. contortus larvae were pipetted into the different compartments of a flat segmented perspex dish, 1.65 cm deep with an internal diameter of 4.4 cm. This dish was then placed on a block of wood directly under the X - ray source (target). The treatment distance i.e. the height of the X - ray source from the larval suspension to be irradiated was 16.7 cm.

As the output of the machine was 1190 rads/minute and the dose required was 40,000 rads the larvae were exposed for exactly 33.61 minutes.

* supplied by Scottish Universities Research Reactor Centre

When two different strains of H. contortus larvae required the same dose and type of irradiation, concentrations of larval suspensions were adjusted, so that they were similar and equal volumes of each suspension were pipetted into perspex tubes/compartments placed diagonally across from each other in the rack/dish thus ensuring as far as possible, that both the strains of larvae received the same treatment.

Blood analysis

Collection and storage of samples

For the analysis of whole blood and plasma, blood samples were obtained in two different vacutainer tubes (Becton - Dickinson, Rutherford, New Jersey, U.S.A.) prepared in a similar way.

a) Whole blood - Two ml of blood was collected in a heparinised vacutainer tube and mixed thoroughly with the anticoagulant by gently inverting the tube three or four times. This sample was used for haematological examinations which were carried out within a few hours of collection.

b) Plasma - Seven ml of blood was withdrawn into a heparinised vacutainer tube and mixed gently with the anticoagulant. After thorough mixing the sample was centrifuged at 2000 rpm for 20 minutes in a MSE centrifuge (Measuring Scientific Equipment, London, England). The separated plasma samples were then transferred using clean Pasteur pipettes into plastic tubes (Luckham Ltd., Victoria Gardens, Sussex) and stored at -20°C.

Haemoglobin (Hb) typing

Before each experiment individual sheep were haemoglobin typed. Separation of haemoglobin was achieved by Multi-Micro bank electrophoresis on cellulose acetate paper strips (Shandon celagram Electrophoresis strips, 78 x 150 mm).

Cellulose acetate strips were saturated with Buffer - A (Pre buffer (Tris 16.1 gm, Disodium EDTA 1.56 gm, Boric acid 0.93 gm, Distilled water 1000 ml)) at a pH of 9.0. After five minutes the paper strips were lightly blotted between clean tissues to remove excess buffer and placed in an electrophoresis tank (Shandon Southern Instruments, Frimly Road, Camberley, Surrey) which was partly filled with 2000 ml of Buffer - B (Barbitone 1.8 gm, Sodium barbitone 10.3 gm, Distilled water 1000 ml) at a pH of 8.5.

Whole blood obtained from individual sheep was haemolysed by mixing a drop of blood with a drop of distilled water on an applicator plate. After careful mixing, the applicator teeth were dipped into the well mixed samples and applied vertically to the cellulose acetate strips about 2.5 cm from the cathode end. The tank containing the strips was then covered, the polarity checked and the samples subjected to a constant voltage of 150 Volts from a Vokam power pack (Shandon Scientific Co.) for 30-40 minutes. The strips were then removed and treated as follows: Soaked for five minutes in 5% aqueous solution of Trichloroacetic acid (TCA), stained for five minutes with Ponceau S in 3% TCA, washed with 5% acetic acid until the background turned white.

Different haemoglobin types have different rates of migration and these are easily recognised in the stained strips. Hb A migrates the farthest and Hb B the least with Hb AB occupying an intermediate position.

Haemoglobin type has been reported to influence resistance to H. contortus infection in sheep (Altaif and Dargie, 1978).

Packed Cell Volume (PCV)

Packed Cell Volume percentages were measured using the microhaematocrit method. Capillary tubes (Gelman - Hawksley, England)

were filled from well mixed blood samples and sealed at one end with 'Cristaseal' (Hawksley and Sons Ltd., Lancing, Sussex). They were then centrifuged for five minutes in a microhaematocrit centrifuge (Gelman - Hawksley Ltd., England) and percentage PCV determined from the scale on a Hawksley microhaematocrit reader.

Haemoglobin (Hb) concentration

Photometric determination of haemoglobin as cyanmethaemoglobin -

In this method blood is treated with potassium ferricyanide solution which oxidises haemoglobin to haemiglobin; haemiglobin is then converted by treatment with cyanide to the stable cyanmethaemoglobin. This compound is measured colorimetrically and the haemoglobin content of the sample determined with the aid of a standard solution.

0.02 ml of blood was pipetted into a clean test tube containing 5.0 ml of Drabkins solution (20 ml of cyanide reagent (Potassium ferri cyanide 0.03 M, Potassium cyanide 0.0387 M, Potassium dihydrogen phosphate 0.0515 M, Sterox 2.5%) diluted to 1 litre with distilled water) mixed well and after three minutes the resulting solution was measured for optical density in a spectrophotometer (Spectronic - 20 Colorimeter/ Spectrophotometer, Bausch and Lomb, Rochester, New York) reading at 540 nm. The optical density of the standard solution (Cyanmethaemoglobin, BDH Chemicals Ltd., Poole, England) was also measured at the same wave length. Hb concentration in grams per 100 ml of the blood was then calculated as follows:

$$\text{gm Hb/100 ml} = \frac{\text{Reading of the test}}{\text{Reading of the standard}} \times 0.25 \times 71.6$$

(0.25 corresponds to the dilution factor and 71.6 to concentration of the standard - mg cyanmethaemoglobin/100 ml).

Total red blood cell (RBC) counts

Total red blood cell counts were determined using an electronic particle counter (Coulter counter, Model Dn, Coulter Electronics Ltd., Herts., England).

Plasma Pepsinogen

Plasma pepsinogen was estimated by a method essentially similar to that of Edwards, Jepson and Wood (1960) in which plasma was incubated for 24 hours at 37°C and at pH 2.0 with BSA (Bovine Serum albumin - No.A - 4503 Albumin, Bovine fraction V, Sigma Chemicals Co. Ltd., Surrey, England) which acted as the substrate. The tyrosine-like phenolic amine acids liberated which are non-precipitable with trichloroacetic acid were determined by the Folin - Ciocalteau reaction. The colour produced when Folin - Ciocalteau's reagent (BDH Chemicals Ltd., Poole, England) was added to the filtrate was measured in a spectrophotometer (Unicam SP 600 - Unicam Ltd., Cambridge, England) and the concentration of the liberated chromogenic substances calculated. Corrections were made for the normal (i.e. not incubated) content of tyrosine-like substances and also for the release of these substances from BSA alone.

The enzyme activity was expressed as milli-units (mU) of tyrosine.

Necropsy procedure

Twenty-four hours before slaughter the animals were starved to enable easy handling of abomasal contents. The animals were killed using a captive bolt pistol and immediately bled out.

Abomasum

A ligature was placed around the pylorus to prevent any loss of contents and the abomasum together with the omasum was carefully removed from the rest of the digestive tract.

The omasum was later removed, the abomasum opened along the greater curvature and the contents carefully emptied into a graduated plastic bucket. The mucosal surface of the abomasum was then washed with lukewarm tap water and the volume of the combined washings and contents adjusted to two litres. After thorough mixing duplicate samples each of 200 ml were taken for estimation of worm populations and 10 ml of formalin was added as preservative to each sample.

The washed abomasum was divided into equal halves and with a butcher's knife the mucosa of the whole of one half was scraped and placed into a honey jar: 200 ml of a mixture of pepsin and hydrochloric acid (800 gm of Pepsin A powder dissolved in four litres of distilled water to which 240 ml of concentrated hydrochloric acid was later added and the whole solution made up to eight litres with distilled water) was then added to the jar which was subsequently incubated at 42°C for six hours. The digest was then made up to two litres and duplicate samples each of 200 ml removed and preserved as described above for the abomasal contents. These samples were examined for parasitic larval stages.

Worm counting and identification

The preserved samples of abomasal contents and digests were mixed well and stained with a few drops of a 45% iodine solution (to 720 gm potassium iodide in 500 ml of warm distilled water, 450 gm iodine crystals were added and made up to one litre with distilled water). After thorough stirring four ml aliquot samples were withdrawn and transferred to petri dishes using a 10 ml straight "sawn off" pipette. The samples were decolourised by the addition of a few drops of a 5% solution of sodium thiosulphate into the petri dishes and examined under a dissecting microscope (Wild Model M5, Heerbrugg, Switzerland). This procedure facilitated counting in that after decolourising with sodium thiosulphate, the worms

retained the stain and were readily visible in the clear background.

Ten aliquot samples were examined and the total numbers of worms present multiplied by 50 to obtain the total number of worms present in the abomasal contents or multiplied by 100 to obtain the total number of larvae present in the mucosal digests.

Statistics

The worm burdens were analysed non-parametrically using the Mann-Whitney U test and probabilities of less than 0.05 were considered significant.

OBSERVATIONS

From the beginning of each experiment blood samples were collected from each lamb or sheep (weekly or twice weekly) and packed cell volumes (PCVs) estimated. In addition, in Experiments 1 and 2 haemoglobin concentrations (Hb), total red blood cell (RBC) counts and plasma pepsinogen values were recorded weekly.

From each animal, weekly or twice weekly faecal samples collected from the rectum were examined for parasite eggs.

At necropsy the abomasum was removed and the numbers of *H. contortus* adults and larval stages present in the abomasal contents and digests of the mucosa recorded.

CHAPTER I

STUDIES DESIGNED TO EXPLORE THE RELATIONSHIP BETWEEN ANTHELMINTICS AND THE IMMUNE RESPONSE TO *HAEMONCHUS CONTORTUS*.

Experiment 1 The effect of anthelmintic removal of worms developing from two doses of irradiated larvae on resistance to subsequent infection

Introduction

This experiment was designed to study the effect of the removal, by levamisole or thiabendazole, of immunising worm burdens developed from infections with irradiated H. contortus larvae on the development of resistance to subsequent challenge infection with normal larvae.

Experimental Design

The design of the experiment is given in Table 1. A total of 21 parasite-naive lambs were used which were 10 months old at the beginning of the experiment. The lambs were divided into three groups of five and one group of six animals based on weight, sex and haemoglobin type. At the beginning of the experiment lambs from Groups I, II and III each received a dose of 10000 γ - irradiated (60 kr) H. contortus larvae orally, followed by a second dose four weeks later. Group IV lambs remained uninfected during this period. Three weeks after each vaccination lambs from Groups I and II each received a dose of thiabendazole and levamisole respectively to remove the worms which had developed from each dose of irradiated larvae. Four weeks after the second vaccination the lambs from all four groups were given 10,000 normal H. contortus larvae and four weeks later all of the animals were killed.

Results

Haematological Estimations

Packed cell volumes (PCVs)

The mean PCVs of all four groups are shown in Figure 1 and the individual values are given in Appendix A.

Table 1

Design of Experiment 1

Group	No. of animals/ group	First vaccination Day 0	Anthelmintic treatment Day 21	Second Vaccination Day 28	Anthelmintic treatment Day 49	Challenge infection Day 56	Necropsy Day 84
I	5	10,000 L_3*	TBZ	10,000 L_3*	TBZ	10,000 L_3	K
II	5	10,000 L_3*	LEV	10,000 L_3*	LEV	10,000 L_3	K
III	5	10,000 L_3*	-	10,000 L_3*	-	10,000 L_3	K
IV	6	-	-	-	-	10,000 L_3	K

* γ-irradiated larvae (60 kr)

K Killed

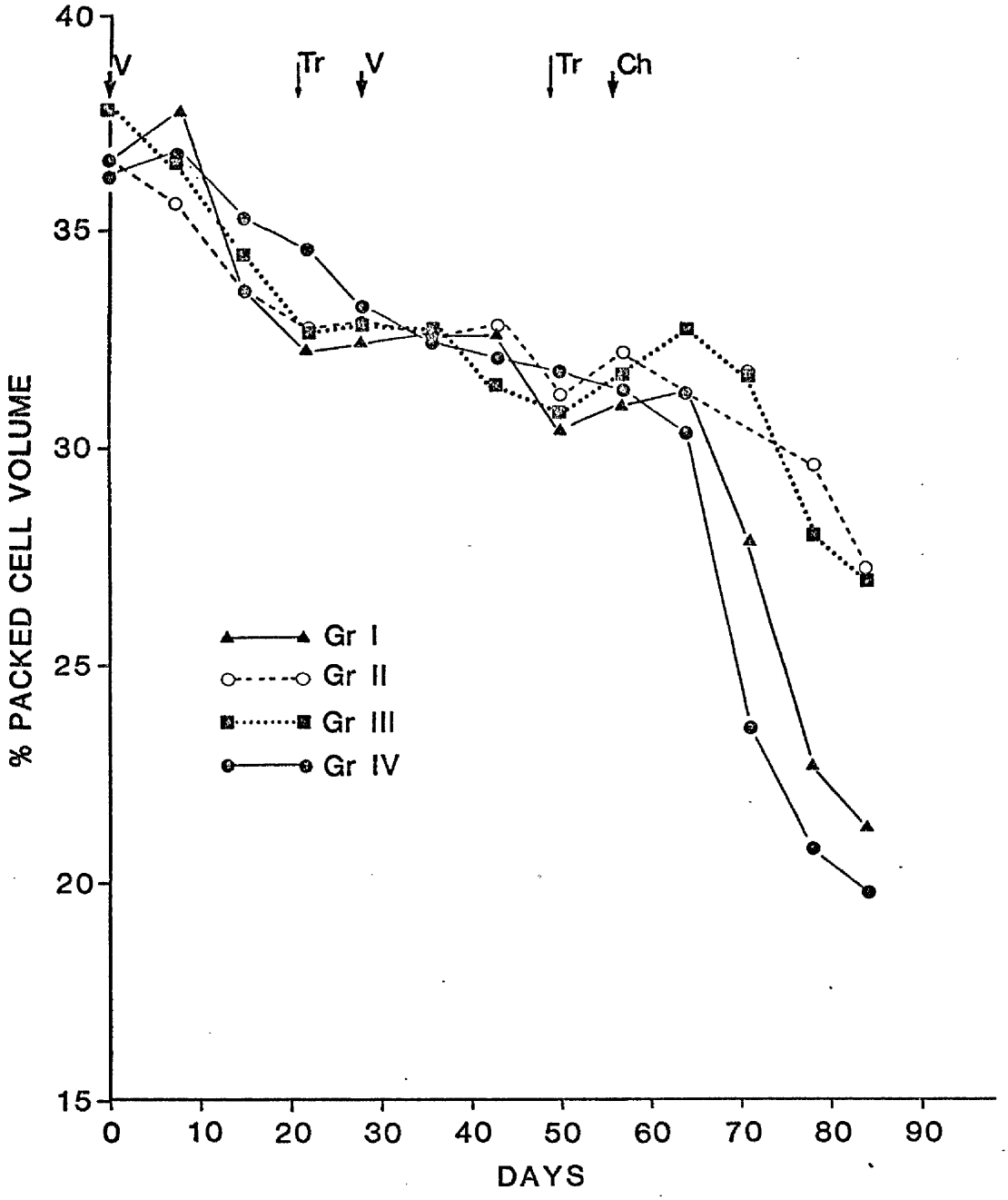

40
35
30
25
20
15
V
Tr
V
Tr
Ch
% PACKED CELL VOLUME
Gr I
Gr II
Gr III
Gr IV
0
10
20
30
40
50
60
70
80
90
DAYS

FIG. 1

The mean PCVs of all four groups of lambs decreased in the period between the first and second vaccinations but subsequently remained at a similar level up to challenge. Between two to four weeks after challenge, all four groups showed a marked decrease in PCV, the lowest values being recorded during the fourth week after challenge: the challenge control group lambs showed the lowest values of around 20% at this time.

Haemoglobin concentrations and RBC counts

The fluctuations in these parameters reflected changes in PCV and the mean and individual values for each group are given in Appendix A.

Plasma pepsinogen values

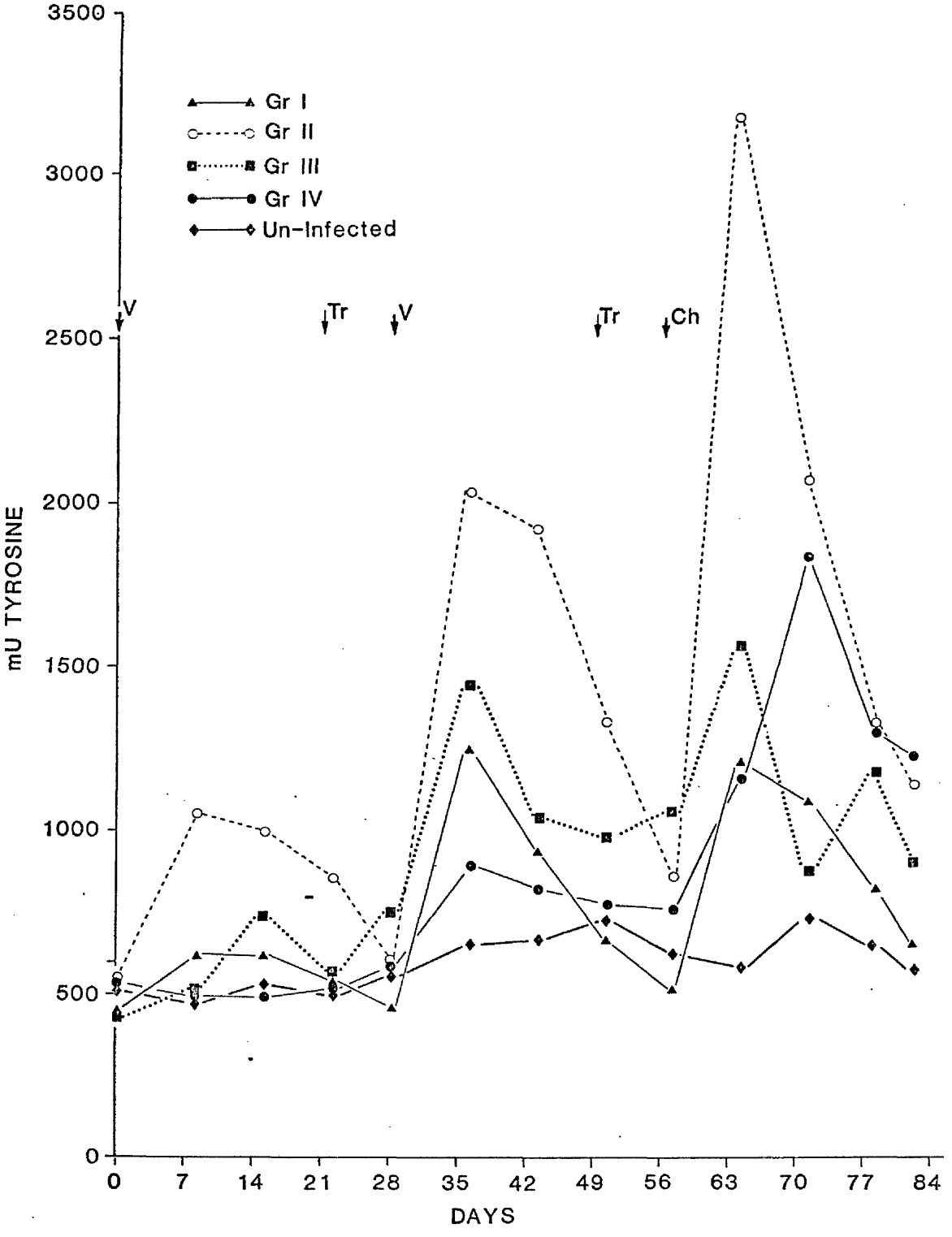
The mean plasma pepsinogen values of all groups expressed as mU of tyrosine are shown in Figure 2 and the individual values are presented in Appendix A.

Following each vaccination there was a rise in plasma pepsinogen values in all of the vaccinated animals and this was more rapid and marked following administration of the second dose of vaccine. After challenge with normal larvae further variable but rapid increases were observed in the vaccinated sheep followed one week later by a rise in the values recorded from the challenge controls. At each stage the vaccinated and levamisole-treated lambs showed the highest mean values reaching a maximum of 3200 mU one week post-challenge.

Parasitological Estimations

Faecal egg counts

Throughout the vaccination period, faecal samples from all of the vaccinated lambs in Groups I, II and III were negative for parasite eggs.


3500
3000
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2000
1500
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500
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mU TYROSINE
Gr I
Gr II
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Un-Infected
V
Tr
V
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0
7
14
21
28
35
42
49
56
63
70
77
84
DAYS

FIG 2

The mean faecal egg counts of all four groups after challenge are given in Figure 3 and the individual values in Appendix A. Peak levels were reached four weeks after challenge with the lambs in the challenge control group showing the highest value of 23200 epg at this time. However, three lambs from Group III and one lamb from Group II had negative faecal egg counts after challenge.

Worm burdens

The mean worm burdens of the lambs in all four groups are given in Table 2 and the individual worm burdens with the details of the various stages recovered are presented in Appendix A. The mean worm burdens of the three vaccinated groups were between 1640-3130, Group I being the highest. However, the mean worm burdens of all of the vaccinated groups were not significantly different from that of the challenge controls (mean 2733). No worms were recovered at necropsy from one lamb in Group II.

Conclusions

The results indicated that, contrary to all previously published reports, the larval vaccine failed to confer protection to subsequent challenge with normal H. contortus larvae.

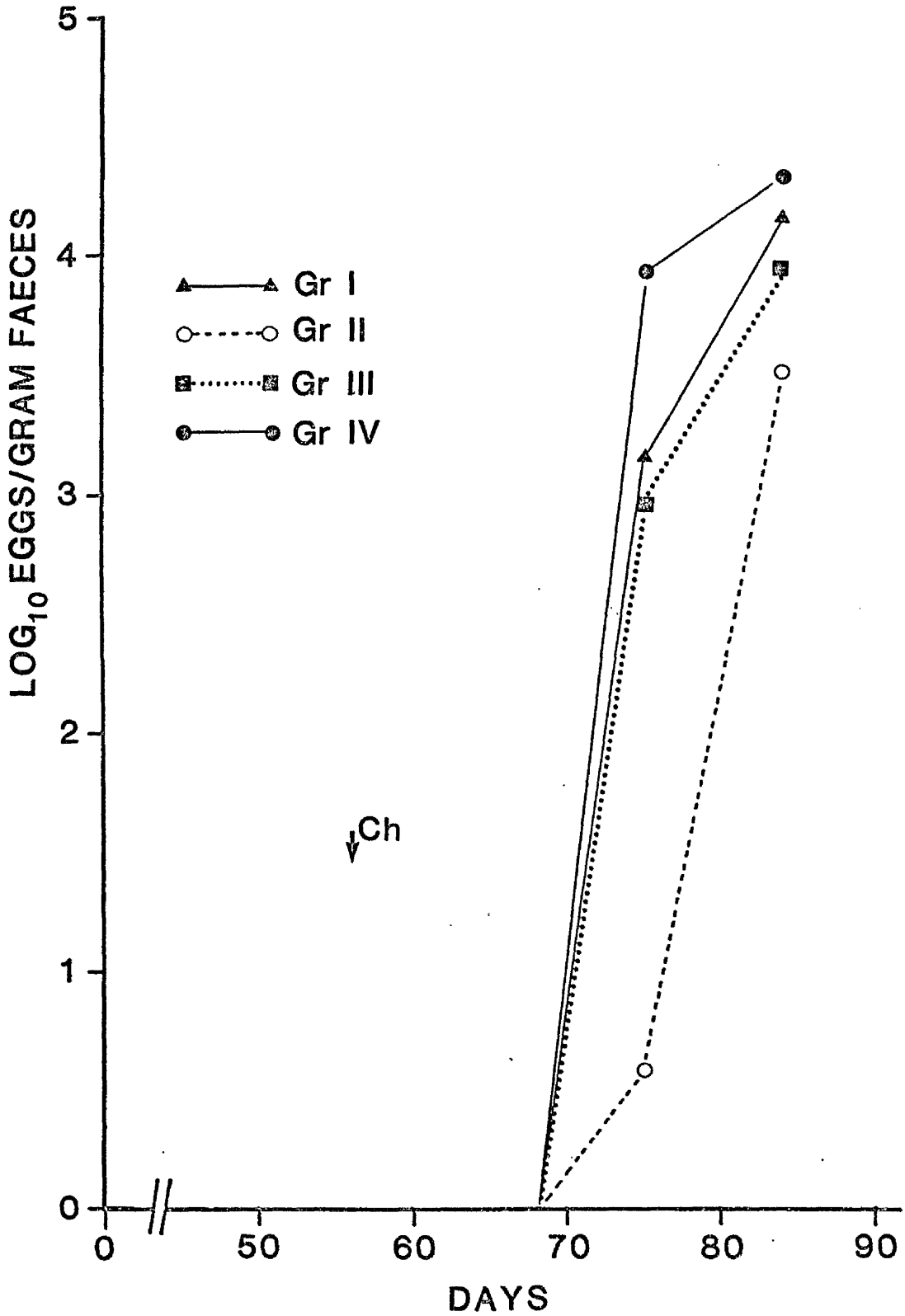

5
4
3
2
1
0
LOG10 EGGS/GRAM FAECES
Gr I
Gr II
Gr III
Gr IV
Ch
0
50
60
70
80
90
DAYS

FIG3

Table 2

The mean (± SE) and range of worm burdens recovered at necropsy of the four groups of lambs

Group	No. of animals/ group	Vaccination/Treatment/Challenge regimen					Worm burdens Mean	Range
I	5	V	TBZ	V	TBZ	C	3130 ± 786	750 - 5000
II	5	V	LEV	V	LEV	C	1640 ± 628	0 - 3800
III	5	V		V		C	2480 ± 1039	650 - 5600
IV	6					C	2733 ± 596	900 - 4750

V - Vaccine
C - Challenge

There was no significant difference between the worm burdens of all four groups.

Experiment 2 The effect of anthelmintic removal of worms developed from an immunising regimen of two doses of irradiated and one dose of normal H. contortus larvae on resistance to subsequent infection

Introduction

This experiment was designed to assess the immunity produced by a regimen of two doses of irradiated larvae followed by one dose of normal larvae, to subsequent challenge with normal larvae. Three groups of lambs were studied, two of which received a single anthelmintic treatment before challenge.

Experimental Design

This experiment commenced at the same time as Experiment I and a total of 21, parasite-naive lambs were used which were 10 months old at the beginning of the experiment. Based on weight, sex and haemoglobin type the lambs were divided into three groups of five and one group of six animals. The design of the experiment is given in Table 3. Lambs from Groups V, VI and VII each received an oral dose of 10,000 γ - irradiated (60 kr) H. contortus larvae followed by a second dose four weeks later. Four weeks after the second dose of irradiated larvae lambs from all three vaccinated groups were infected with 10,000 normal H. contortus larvae. Three weeks after this infection the animals in Groups V and VI received a dose of thiabendazole and levamisole respectively to remove all the worms which had developed from the two doses of irradiated and single dose of normal larvae. One week after anthelmintic treatment, the lambs from Groups V, VI and VII were challenged at the same time as a group of uninfected lambs (Group VIII) with 10,000 normal H. contortus larvae. Four weeks later all of the animals were killed.

Table 3

Design of Experiment 2

Group	No. of animals/ group	First vaccination Day 0	Second vaccination Day 28	Infection Day 56	Anthelmintic treatment Day 77	Challenge infection Day 84	Necropsy Day 112
V	5	10,000 L_3*	10,000 L_3*	10,000 L_3	TBZ	10,000 L_3	K
VI	5	10,000 L_3*	10,000 L_3*	10,000 L_3	LEV	10,000 L_3	K
VII	5	10,000 L_3*	10,000 L_3*	10,000 L_3	-	10,000 L_3	K
VIII	6	-	-	-	-	10,000 L_3	K

* γ-irradiated larvae (60 kr)
K Killed

Results

Haematological Estimations

Packed cell volumes (PCVs)

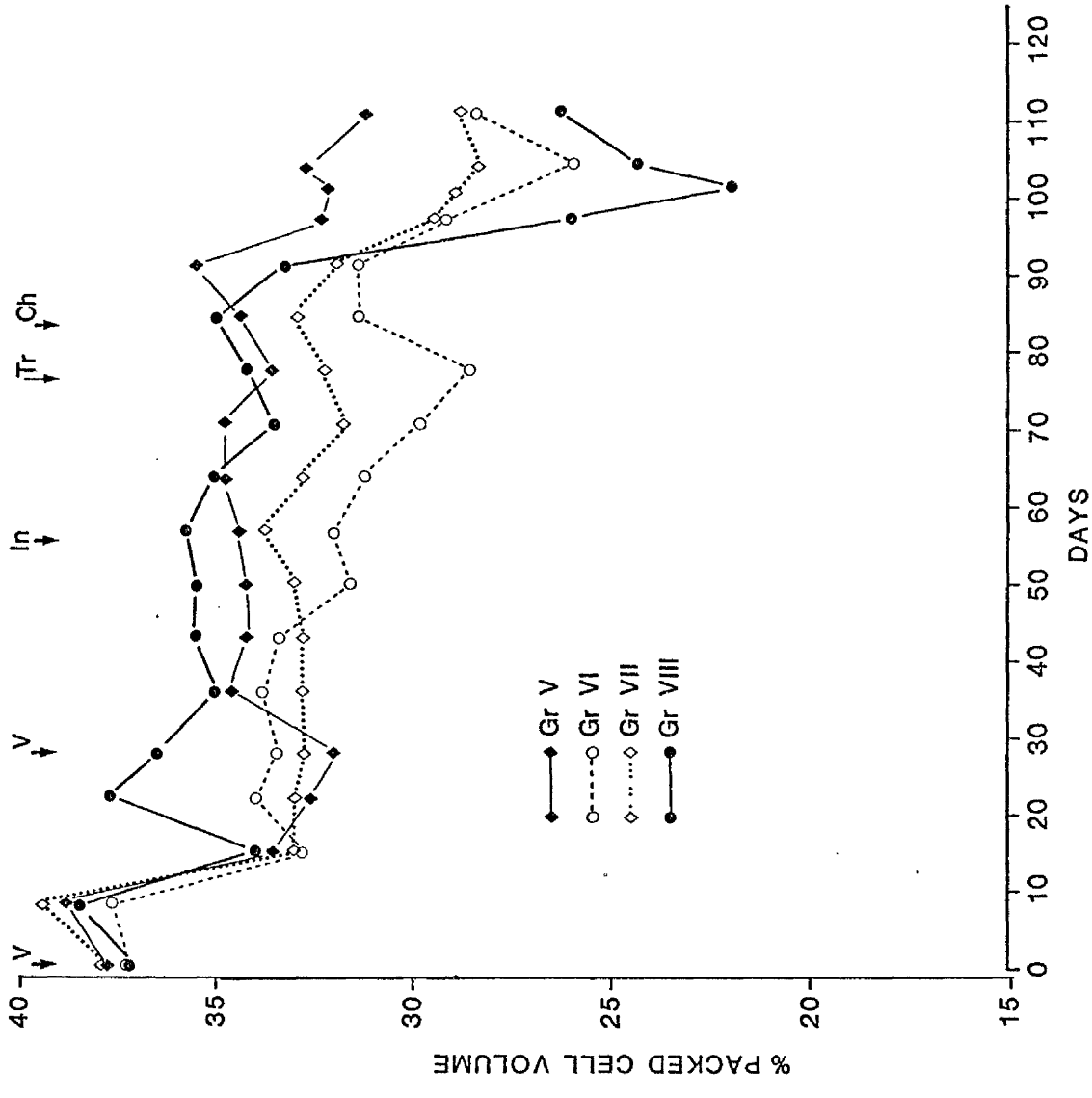
The mean PCVs of all four groups are shown in Figure 4 and the individual values are presented in Appendix A. After the first vaccination the mean PCVs of the three vaccinated groups decreased and subsequently remained at a similar level until infection with normal larvae: following this, Groups VI and VII showed further decreases in PCV while the mean PCVs of the Group V lambs remained at a fairly steady level. After challenge the PCVs of the control lambs in Group VIII showed a marked decrease reaching a mean of 22% three weeks later. Over the same period the mean PCVs of the other three groups also decreased to between 26-32%.

Haemoglobin concentrations and RBC counts

The mean and individual values are given in Appendix A. The fluctuations in these parameters were similar to those of the PCVs.

Plasma pepsinogen values

The mean plasma pepsinogen values of all four groups expressed as mU tyrosine are shown in Figure 5 and the individual values are presented in Appendix A. Following each vaccination the plasma pepsinogen values rose in the vaccinated groups and further increases in these values occurred after infection with normal larvae. Marked increases in plasma pepsinogen levels occurred in all groups following challenge. The highest values were recorded from the Group VII lambs which reached a mean maximum of 3800 mU approximately one week after challenge.


% PACKED CELL VOLUME
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Gr V
Gr VI
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V
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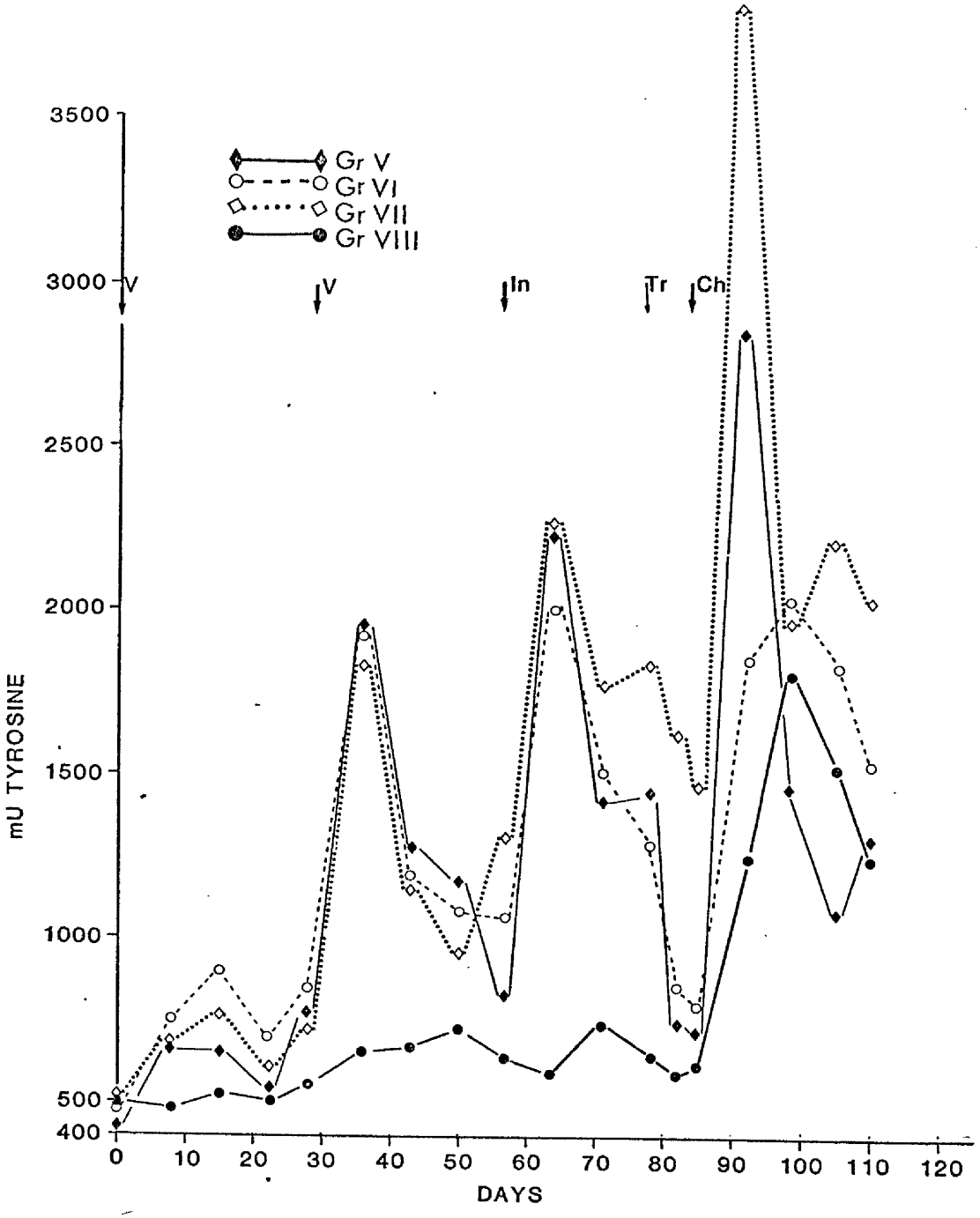
100
110
120

FIG 4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0
10
20
30
40
50
60
70
80
90
100
110
120
DAYS

FIG 5

Parasitological Estimations

Faecal egg counts

Throughout the vaccination period faecal examinations from all three vaccinated groups proved negative. The mean faecal egg counts of all four groups after challenge are shown in Figure 6. After the infection with normal larvae the Group VII lambs had positive counts from day 84 onwards while the Group VI lambs showed positive counts only after challenge. During the whole experimental period only one lamb from Group V had positive egg counts and these appeared after challenge. The highest egg counts were found in the Group VIII controls four weeks after challenge.

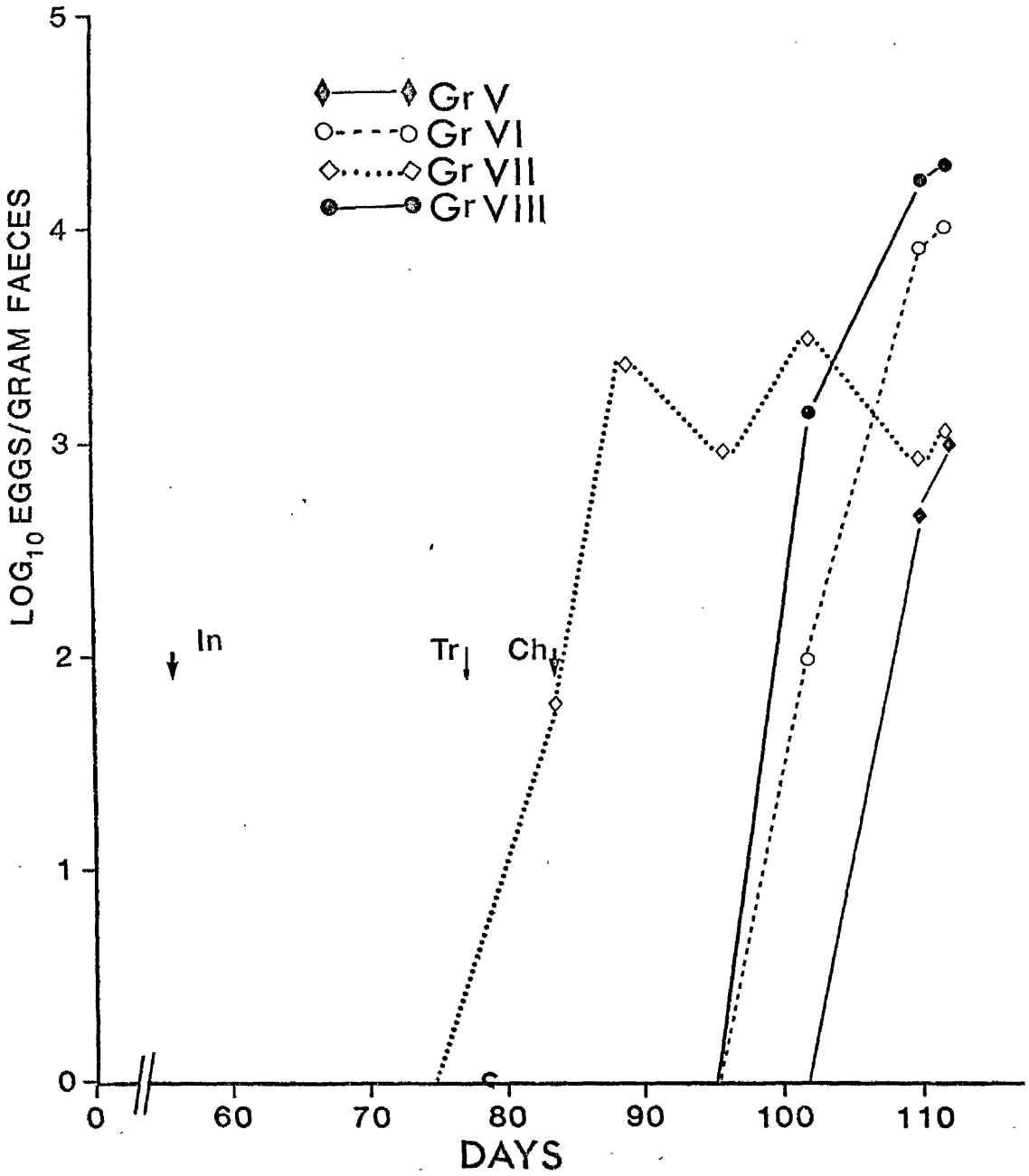
Worm burdens

The mean worm burdens of the lambs in all four groups are given in Table 4 and the individual worm burdens together with the details of the various stages recovered are presented in Appendix A. Although there was no significant difference between the worm burdens of any of the vaccinated groups and the worm burdens of the challenge controls as assessed by the Mann-Whitney U test, the Group VII lambs had the lowest mean worm burden of 838 compared with a mean worm burden of 2050 in the challenge controls.

One lamb from Group VIII had no worms at necropsy although it had shown consistently positive egg counts after challenge.

Conclusions

In this experiment the results suggested that the group of lambs which received the two doses of irradiated larvae followed by a dose of normal larvae had developed some immunity against challenge. However, because of the high SE this was not statistically significant. In addition, there was the suggestion that the anthelmintic removal of the immunising worm burdens allowed increased


Gr V
Gr VI
Gr VII
Gr VIII
LOG10 EGGS/GRAM FAECES
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In
Tr
Ch
0
60
70
80
90
100
110
DAYS

FIG 6

Table 4

The mean (± SE) and range of worm burdens recovered at necropsy of the four groups of lambs

Group	No. of animals/ group	Vaccination/Treatment/Challenge regimen					Worm burdens Mean	Range
V	5	V	V	I	TBZ	C	1530 ± 444	400 - 2850
VI	5	V	V	I	LEV	C	2470 ± 537	1450 - 4500
VII	5*	V	V	I		C	838 ± 276	150 - 1500
VIII	6					C	2050 ± 633	0 - 4250

* one lamb died of urolithiasis early in the course of the experiment

V - Vaccine
I - Infection
C - Challenge

There was no significant difference between the worm burdens of all four groups.

establishment of the challenge infection but the results were again not statistically significant.

DISCUSSION

Following the successful vaccination of calves against the bovine lung worm Dictyocaulus viviparus using X-irradiated infective larvae, Jarrett and his colleagues (Jarrett et al, 1959) attempted to immunise sheep with H. contortus larvae. They found that seven month old lambs were protected against a challenge consisting of 8000 larvae following oral administration of a single dose of 10,000 X-irradiated larvae. From these experiments they also suggested that the optimal level of X-irradiation was between 40 kr and 60 kr. Later the same group of workers (Jarrett et al, 1961) showed that following double vaccination with 10,000 L_3 irradiated at 40 kr, lambs aged eight months were able to resist a challenge infection consisting of 50,000 normal H. contortus larvae; the immunity conferred was highly significant in that the worm burdens of the vaccinated animals were reduced by 99% compared with those of control sheep. Bitakaramire (1966) confirmed these findings and showed that the immune response was directed against third and fourth larval stages of the challenge infection. Around the same time, it was shown that lambs less than seven months of age could not be immunised by the administration of X-irradiated larvae (Urquhart, Jarrett, Jennings, McIntyre, Mulligan and Sharp, 1966a,b) and later studies using γ - irradiated larvae also failed to produce protection in this age group of lambs (Duncan, Smith and Dargie, 1978; Smith and Angus, 1980).

Working with H. contortus in Australia Mulligan, Gordon, Stewart and Wagland (1961) found it necessary to use an X - ray dose of 60 kr to produce a degree of attenuation comparable to that produced by 40 kr in Scotland by Jarrett and his colleagues (1961) although the quality of the radiation and the dose rate

were similar in both experiments. They attributed this variation in the radiation dose required for the necessary level of attenuation to differences in radiosensitivity and inherent pathogenicity of the two strains of H. contortus larvae used.

In a ^{60}Co dose titration experiment in this laboratory Benitez-Usher found that irradiation at 60 kr was more suitable than 40 kr (Smith and Christie, 1978). This apparent variation in the required radiation dose using X-ray and γ- ray sources may have been due to differences in the relative biological efficiency (RBE) of the two types of radiation since, Kassai, Fitzpatrick and Mulligan (1966) using the rat/Nippostrongylus brasiliensis host-parasite system, have shown that the RBE of γ - rays is less than that of X - rays in the dose range where attentuation is manifested. Using the ^{60}Co source to attenuate H. contortus larvae Benitez-Usher et al (1977) successfully protected nine month old lambs against a challenge with 10,000 normal larvae following double vaccination with 60 kr irradiated larvae administered at monthly intervals.

Smith and Christie (1978) also found that when using γ - irradiation, 60 kr was a suitable dose which produced the required level of attenuation and they successfully immunised sheep with H. contortus larvae irradiated at this level. In later experiments many workers (Duncan, Smith and Dargie, 1978; Smith and Christie, 1979; Smith and Angus, 1980) also found that a high degree of protection was conferred against challenge infection in sheep immunised using this regimen.

However, from the results obtained in the two experiments reported here it was evident that double vaccination of mature lambs with 10,000, 60 kr γ - irradiated larvae failed completely to confer protection against a challenge infection consisting of

10,000 normal larvae. In both experiments there was no statistically significant difference between the mean worm burdens of the control groups, and the vaccinated groups, whether treated with anthelmintic or not. The only clear evidence that the immune response was operating at some level was the fact that faecal samples from four immunised lambs in the first experiment and four in the second experiment were consistently negative for parasite eggs after the challenge infection. Faecal egg output is perhaps the most sensitive index of immunity (Jarrett and Urquhart, 1971) and since all of these lambs except one, had reasonable worm burdens it is likely that the immune response was responsible for this effect.

There was no evidence of any error which could account for this failure of a previously successful vaccination regimen either during the irradiation procedure or during the course of the experiment. The ^{60}Co source was recalibrated and was found to be emitting the expected radiation dose. Also the lambs in both experiments were over seven months of age and therefore immunologically 'mature' at the beginning of the experiments. There was therefore no obvious explanation for the failure of the vaccine to confer protection but one possible factor considered was that the H. contortus larvae, during regular passage in sheep under laboratory conditions, had developed some degree of radiosensitivity or radioresistance. The latter however was unlikely since no eggs were found in any of the vaccinated lambs during the whole of the vaccination period. Another possible explanation was that the larvae had become less immunogenic.

Shortly after these experiments were completed we found from discussions with other workers in Glasgow and Edinburgh (Duncan, personal communication; Smith, personal communication) that they also had recently experienced difficulty vaccinating lambs successfully

with γ - irradiated H. contortus larvae subjected to 60 kr. It was subsequently established that the strain of parasite used by both workers and by the writer was from the same source.

Since this situation had developed it was thought best to abandon the original objective of these studies and instead to attempt to determine the reason, or reasons, underlying the failure of the vaccination regimen. The subsequent experiments in this section are therefore concerned with this objective.

SUMMARY

The two experiments described in this chapter were designed to study the effect of anthelmintic treatment on the immune response stimulated by two doses of Haemonchus contortus larvae γ - irradiated at 60 kr.

Unfortunately no conclusions could be drawn from this study due to the unexpected failure of the vaccine to confer an effective immunity. There were no obvious reasons for this result and in consequence it was decided to abandon the original objective and instead to investigate the cause or causes underlying the failure of the irradiated larval vaccine.

CHAPTER II

STUDIES ON RADIATION ATTENUATION OF *HAEMONCHUS CONTORTUS* LARVAE AND THEIR IMMUNOGENIC POTENTIAL.

Experiment 3 A γ - irradiation dose titration study using a laboratory maintained and a field strain of H. contortus

Introduction

The principle involved in radiation attenuated helminth vaccines depends on achieving a dose of radiation which is sufficient to reduce the pathogenic effect of the parasite without destroying its immunogenicity.

In the first two experiments reported in this section the irradiated H. contortus larval vaccine which had been used successfully in this laboratory for nearly 20 years failed to immunise lambs against challenge infection with normal larvae. During this period the strain of H. contortus used was one which had been maintained by sub-inoculation of susceptible lambs. All the technical aspects of these two experiments were thoroughly checked and found to be satisfactory and the possibility of acquired radioresistance of the parasite was considered improbable since H. contortus eggs were not detected in any of the faeces of the vaccinated animals.

One possible explanation for the vaccine failure was that the larvae had become radiosensitive and therefore were overirradiated when exposed to 60 kr γ - irradiation. They might therefore have failed to develop in sufficient numbers to an immunogenic stage.

In an attempt to examine that the strain of H. contortus maintained in this laboratory had become radiosensitive an irradiation dose titration experiment was designed to compare the radiosensitivity of the laboratory-maintained strain of H. contortus (Glasgow strain) with an unrelated field strain of H. contortus recently isolated from sheep in the south of England (Cambridge strain). Irradiation was carried out at two levels i.e. 40 kr and 60 kr.

The criterion used to assess radiosensitivity in this experiment was the percentage establishment or 'take' of single doses of irradiated

larvae of each strain at both radiation levels, compared with that of normal larvae in appropriate controls.

Experimental Design

The design of the experiment is given in Table 5. Eighteen, 12 week old parasite-naive Scottish Blackface lambs were divided into six equal groups on the basis of body weight, sex and haemoglobin type. At the beginning of the experiment the lambs in Groups I and II each received 10,000 Cambridge strain *H. contortus* larvae irradiated at 40 kr and 60 kr respectively in a ^{60}Co source while the Group III lambs each received 10,000 normal Cambridge strain larvae. The lambs in Groups IV and V each received 10,000 Glasgow strain larvae similarly irradiated at 40 kr and 60 kr respectively while the Group VI lambs each received 10,000 normal Glasgow strain larvae. Twenty-six days after infection all of the lambs were killed.

Results

Haematological Estimations

Packed cell volumes (PCVs)

The mean PCVs of all six groups are shown in Figure 7 and individual values are presented in Appendix A. A decrease in the mean PCVs of the control lambs in Groups III and VI was evident from day eight and these values reached low levels by the third week after infection. For example, Group VI lambs which received normal Glasgow strain larvae had mean values of around 14% during this period compared with a mean of 23% in the Group III lambs infected with normal Cambridge strain larvae.

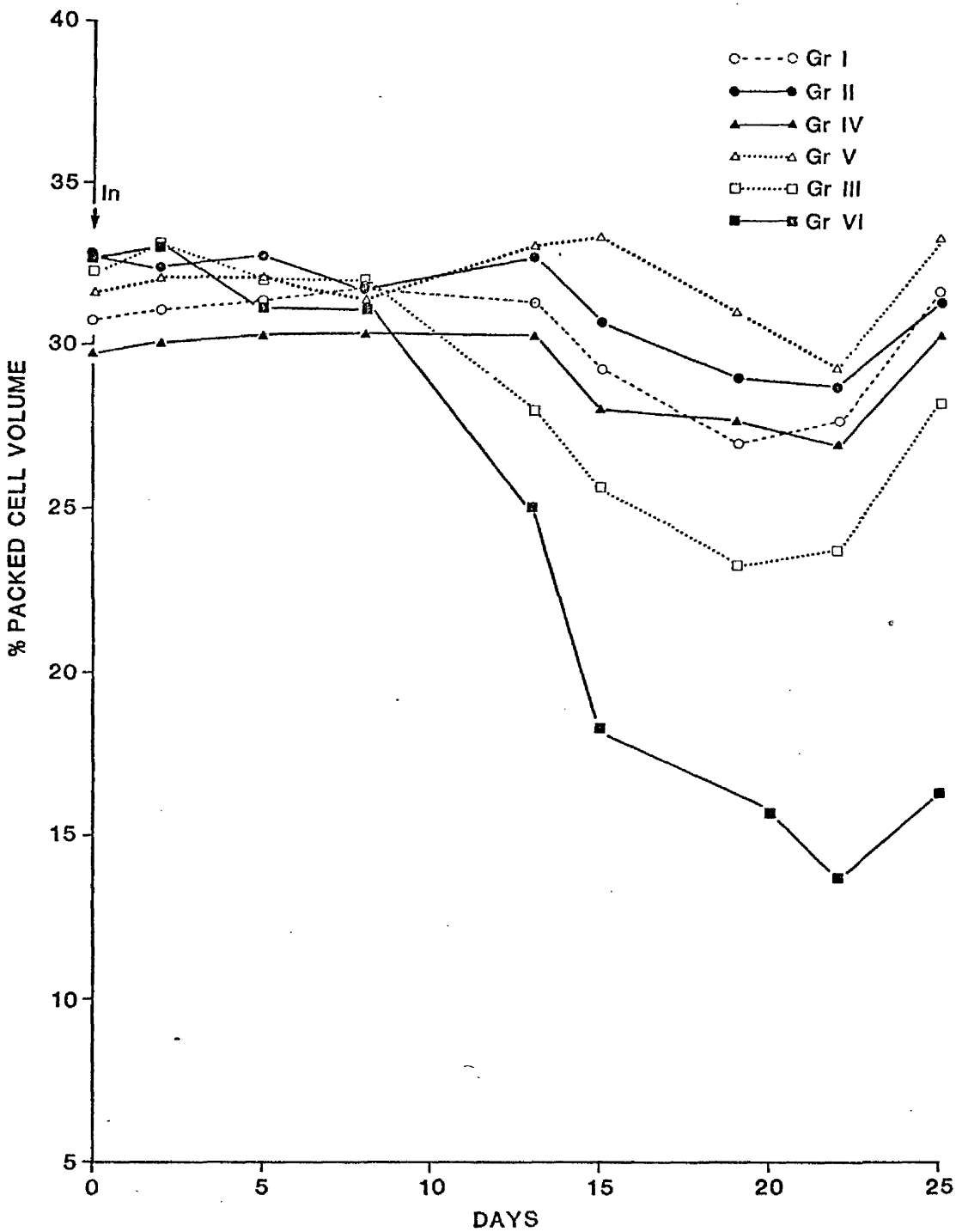
The groups of lambs given irradiated larvae of either strain showed slight decreases in mean PCVs during the second week of infection. Those which received larvae irradiated at 40 kr (Groups I

Table 5

Design of Experiment 3

Group	No. of animals/ group	H. contortus strain	Dose of ^{60}Co irradiation	Infection Day 0	Necropsy Day 26
I	3	Cambridge - Field	40 kr	10,000 L_3	K
II	3		60 kr	10,000 L_3	K
III	3		-	10,000 L_3	K
IV	3	Glasgow - Laboratory	40 kr	10,000 L_3	K
V	3		60 kr	10,000 L_3	K
VI	3		-	10,000 L_3	K

K Killed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In
% PACKED CELL VOLUME
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Gr I
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Gr III
Gr VI

FIG 7

and IV) had slightly lower values than the groups given larvae irradiated at 60 kr (Groups II and V). By day 25 however, the mean PCVs of all four groups given irradiated larvae had returned to their original values.

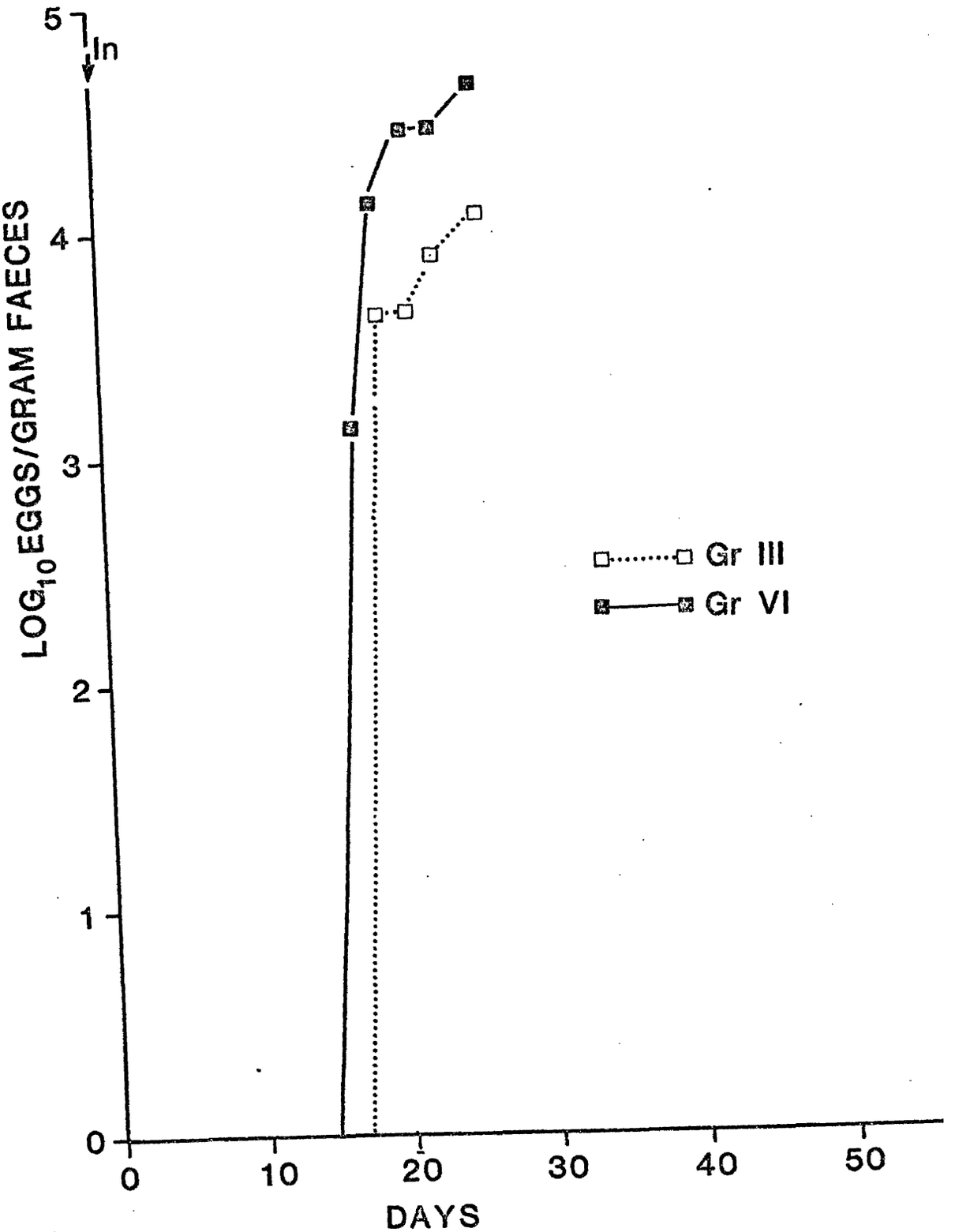
Parasitological Estimations

Faecal egg counts

The mean faecal egg counts of all six groups are given in Figure 8 and the individual values are presented in Appendix A. Faecal samples from the four groups of lambs given irradiated larvae were consistently negative. The Group VI lambs which received normal Glasgow strain larvae had positive egg counts from day 17 onwards while the Group III lambs which received normal Cambridge strain larvae became positive from day 19. Both of these groups showed peak egg counts on day 26 after infection but at all times the Group VI lambs had considerably higher mean egg counts than those in Group III. For example, the mean egg counts of Groups VI and III on day 26 were 46067 epg and 12300 epg respectively.

Worm burdens

The mean worm burdens of the six groups of lambs are given in Table 6 and the individual burdens in Appendix A. Except in Group IV which received 40 kr Glasgow larvae and had a small number of adult male worms all the other lambs given irradiated larvae harboured only stunted female worms. In control Groups III and VI which received normal larvae both adult male and female worms were recovered. The 'take' of normal Glasgow larvae was higher than that of the Cambridge strain, the group mean worm burdens at necropsy being 2400 and 1533 respectively. When examined statistically there was no significant difference between the worm burdens of Groups I and II which received irradiated Cambridge strain larvae and their controls in Group III but the worm burdens of Groups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DAYS
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FIG 8

Table 6

The mean (± SE) and range of worm burdens recovered at necropsy of the six groups of lambs

Group	No. of animals/ group	Infection/Irradiation dose		Worm burdens	
				Mean	Range
I	3	Cambridge strain	(10,000 L_3 - 40 kr	900 ± 293	350 - 1350
II	3		(10,000 L_3 - 60 kr	933 ± 233	500 - 1300
III	3		(10,000 L_3 - normal	1533 ± 387	1050 - 2300
IV	3	Glasgow strain	(10,000 L_3 - 40 kr	767 ± 219	500 - 1200
V	3		(10,000 L_3 - 60 kr	650 ± 208	350 - 1050
VI	3		(10,000 L_3 - normal	2400 ± 427	1900 - 3250

IV and V which received irradiated Glasgow strain larvae were significantly lower (P = 0.05) compared with their Group VI controls.

Conclusions

From this irradiation dose titration experiment using two different levels of γ - irradiation the following differences in behaviour of the laboratory and field strains of H. contortus were evident. Using normal larvae more worms appeared to establish from the laboratory strain than from the field isolate, the percentage establishment being 24% for the Glasgow strain and 15% for the Cambridge strain. The Glasgow strain also appeared to be more pathogenic as all three lambs infected with normal larvae developed anaemia with PCVs below 18%. This was due in part to the fact that more worms were present in these lambs but also it appeared from a comparison of relative worm burdens and haematocrit values that the blood loss per individual worm was greater in this group. Poeschel and Todd (1972), using five different H. contortus isolates from sheep, have shown that different strains differ in their disease producing capacity. The fecundity of the female worms calculated from egg output and worm burdens at necropsy was also higher for the Glasgow strain than for the Cambridge strain.

When the percentage establishment of the irradiated larvae of each strain was compared relative to their appropriate control group given normal larvae, the establishment of irradiated larvae from the Glasgow strain was much lower than the establishment of the irradiated larvae from the Cambridge strain, i.e. at 40 kr and 60 kr, an establishment of 32% and 27% compared with 59% and 61% respectively. This variation in establishment may be due to differences in radiation susceptibility since it has been shown previously (Mulligan et al, 1961) that different isolates of H. contortus required different levels of radiation to produce a similar degree of attentuation

based on worm establishment.

In this study the percentage establishment of larvae irradiated at 40 kr and 60 kr was similar for each of the two isolates and for the Glasgow isolate was essentially the same as those reported previously (Jarrett et al, 1959; Urquhart et al, 1966a).

Despite the limitations of small numbers of animals the results of this experiment clearly showed that, the Glasgow strain of H. contortus had not developed any radiosensitivity detectable by worm establishment and further vaccination studies were therefore designed to investigate the possibility that the Glasgow strain had lost its immunogenicity after repeated laboratory passage.

Experiments 4 and 5 Vaccination studies in immunologically immature and mature lambs with laboratory and field strains of H. contortus

Introduction

Since no obvious signs of altered radiosensitivity were evident from the dose titration experiment vaccination studies were designed to compare the immunogenicity of the Glasgow and Cambridge strains of H. contortus larvae.

Basically, two experiments were carried out using both strains of larvae in five month and 10 month old lambs respectively. These were simple double vaccination and challenge experiments in each case using a vaccine consisting of 10,000 γ - irradiated larvae given at monthly intervals followed by a challenge infection with 10,000 normal larvae one month later. Irradiation was carried out at 60 kr in a ^{60}Co source since the preliminary dose titration experiments in lambs had indicated similar worm establishment when larvae were irradiated at either 40 kr or 60 kr.

Experiment 4 A vaccination study in young lambs

Experimental Design

The experimental design is shown in Table 7. A total of 12, five month old parasite naive lambs were used. The lambs were divided into four equal groups based on weight, sex and haemoglobin type. At the beginning of the experiment lambs from Groups VII and IX each received an oral dose of 10,000 irradiated (60 kr) H. contortus larvae: Group VII received the Glasgow strain and Group IX the Cambridge strain. This was followed by a similar dose of vaccine four weeks later. Groups VIII and X remained uninfected during this period. Four weeks after the second vaccination lambs from Groups VII and VIII each received a challenge infection consisting

Table 7

Design of Experiment 4

Group	No. of animals/ group	H. contortus strain	First Vaccination Day 0	Second Vaccination Day 28	Challenge infection Day 56	Necropsy Day 84
VII	4	Glasgow	10,000 L_3*	10,000 L_3*	10,000 L_3	K
VIII	4	Glasgow	-	-	10,000 L_3	K
IX	4	Cambridge	10,000 L_3*	10,000 L_3*	10,000 L_3	K
X	4	Cambridge	-	-	10,000 L_3	K

* 60 kr γ-irradiated larvae
K Killed

of 10,000 normal Glasgow strain larvae while lambs from Groups IX and X each received a similar challenge consisting of 10,000 normal Cambridge strain larvae. Four weeks after the challenge infection all 12 lambs were killed.

Results

Haematological Estimations

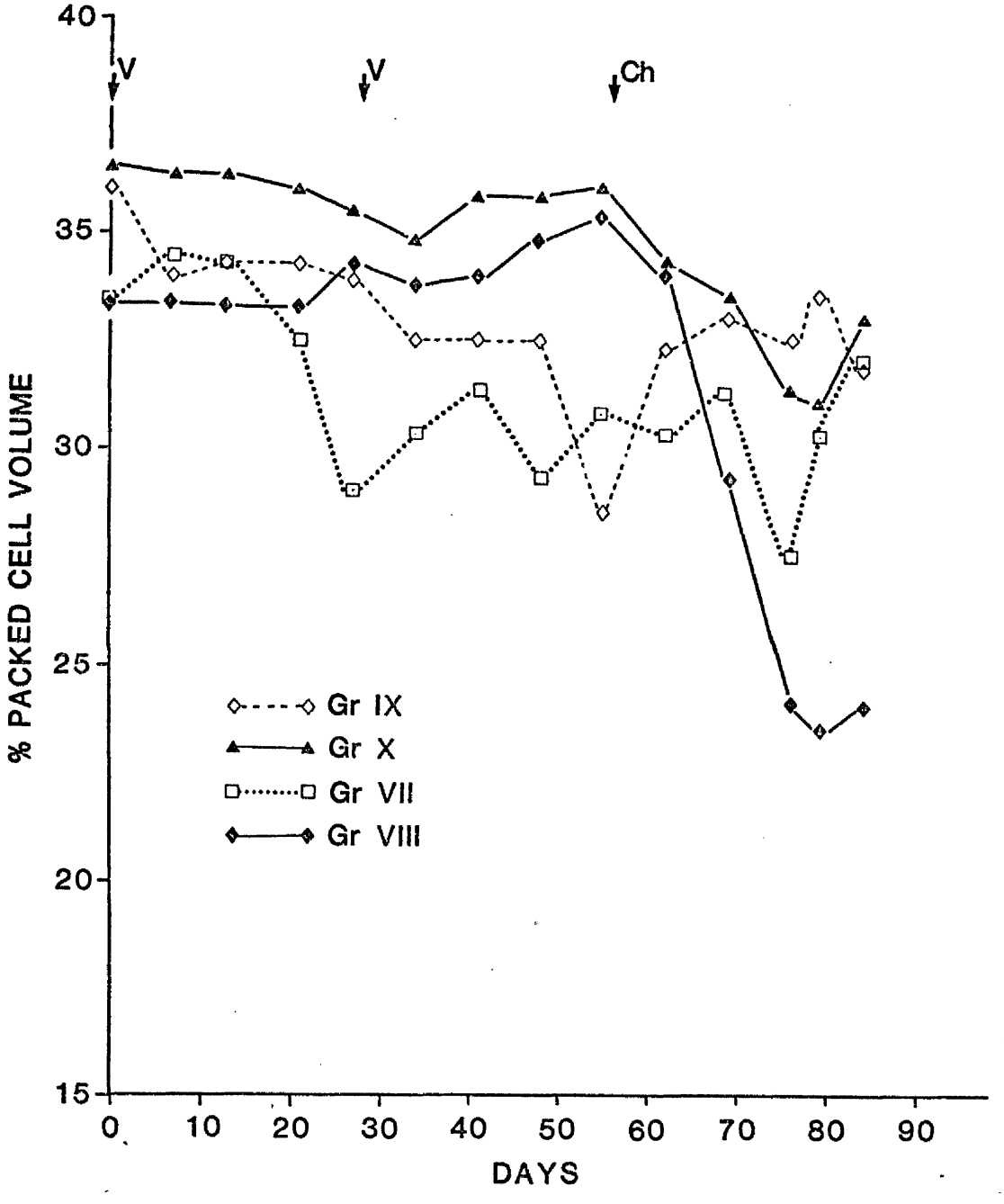
Packed cell volume percentages (PCVs)

The mean PCVs of the four groups of lambs are given in Figure 9 and the individual values are presented in Appendix A. Group VII lambs which were vaccinated with Glasgow strain of larvae showed a decrease in mean PCV around the time of the second vaccination while the Group IX Cambridge vaccinated lambs showed a very gradual decline until the time of challenge. By three weeks after challenge the mean PCV of the Group VIII lambs which received normal Glasgow larvae had fallen from around 34% to 24%, while the Group X lambs which were challenged with the Cambridge strain showed a less marked decrease from 36% to 31%. The PCVs of the Group VII lambs showed a slight decrease during the third week after challenge but recovered to almost pre-vaccination levels by the end of the experiment: Group IX lambs showed little change in mean PCV after challenge.

Parasitological Estimations

Faecal egg counts

The mean faecal egg counts of all four groups are given in Figure 10 and the individual values are presented in Appendix A. During the period of vaccination until two weeks after challenge examination of faeces from lambs in Groups VII and IX were consistently negative for nematode eggs. Twenty days after challenge positive egg counts were recorded in all four groups. Group VIII, the Glasgow controls had the highest mean egg counts at all times whereas the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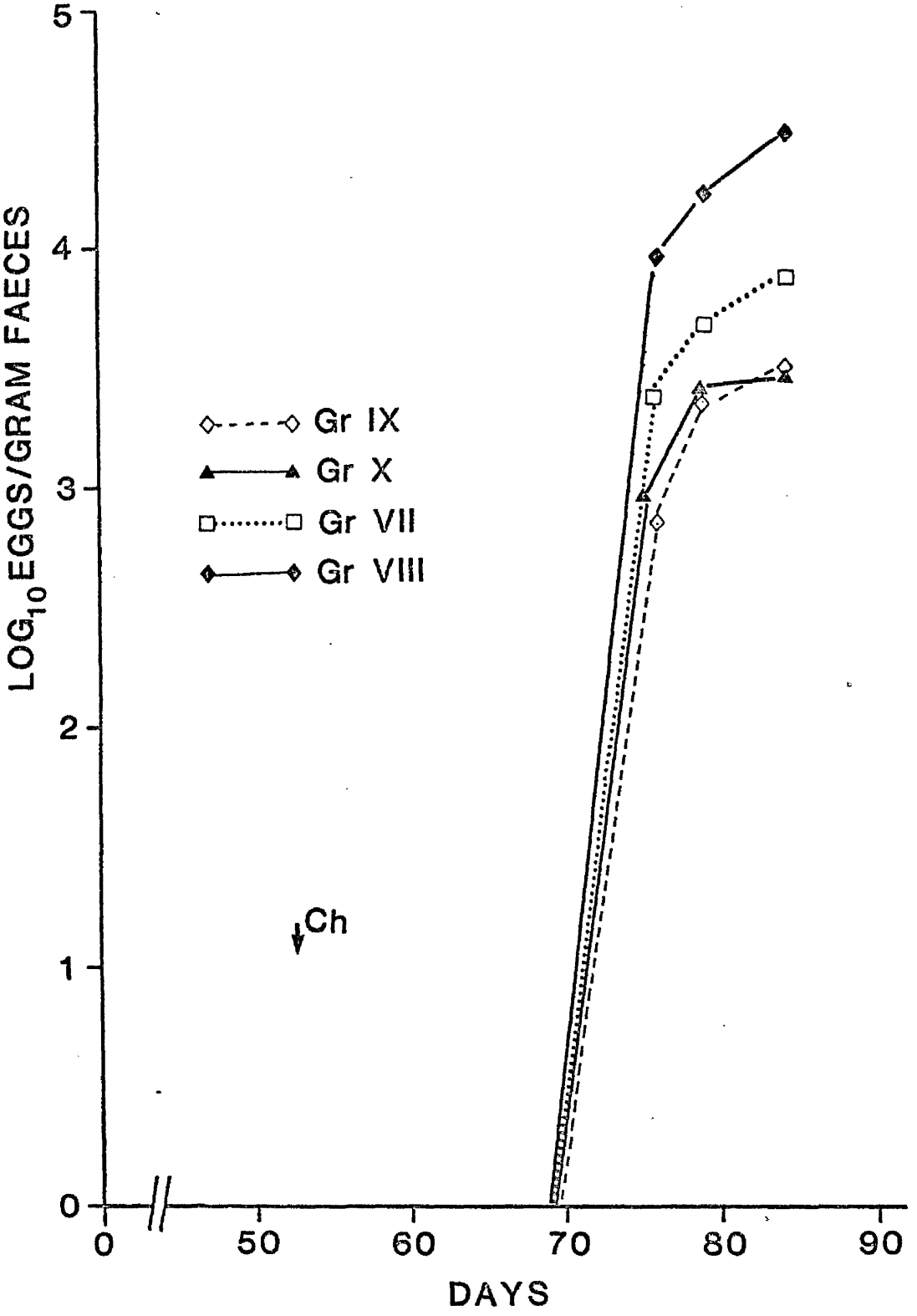
DAYS

FIG 9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LOG10 EGGS/GRAM FAECES
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Gr X
Gr VII
Gr VIII
Ch
0
50
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70
80
90
DAYS

FIG 10

mean egg counts of both Cambridge vaccinates and controls were similar.

Worm burdens

The mean worm burdens of all four groups are given in Table 8 and the individual worm burdens with the details of the various stages recovered are presented in Appendix A. The Glasgow control lambs harboured the highest mean worm burden of 2750 adult worms compared with 1675 in the Glasgow vaccinates but no worms were recovered from one lamb in the latter group. The Cambridge control lambs had a mean worm burden of 1263 at necropsy but in all cases a considerable percentage (range 7-68%) of L_4 and L_5 stages were present. The mean worm burden of the Cambridge vaccinates was 675 and although they too harboured immature stages the percentage of these was smaller than the controls (range 15-43%). The worm burdens of both groups of vaccinated lambs did not differ significantly from those of their appropriate controls.

Conclusions

In this experiment lambs aged five months were used. This was, in fact, not ideal since previous reports have clearly shown that lambs do not respond fully to vaccination until over seven months of age. However, for logistic reasons these younger lambs were vaccinated.

As judged by the results of a previous study with this age of lambs (Urquhart, Jarrett, Jennings, McIntyre and Mulligan, 1966b) one should expect a reduction in worm burdens of the order of 66% significant when measured by the Mann-Whitney U test ($P = 0.028$). The result of the present experiment did not confirm this since the worm burdens of the vaccinated lambs were not significantly different from those of the control groups.

Table 8

The mean (± SE) and range of worm burdens recovered at necropsy of the four groups of lambs

Group	No. of animals/ group	Vaccination/Challenge regimen		Worm burdens Mean	Range
VII	4	Glasgow strain	(Vaccine/Vaccine/Challenge	1675 ± 950	0 - 4000
VIII	4	Glasgow strain	(Challenge	2750 ± 1137	100 - 3500
IX	4	Cambridge strain	(Vaccine/Vaccine/Challenge	675 ± 161	350 - 1000
X	4	Cambridge strain	(Challenge	1263 ± 403	300 - 2250

The results of the faecal egg counts and PCVs were similar to those reported in the previous experiment and confirmed the higher infectivity of the Glasgow strain.

Experiment 5 A vaccination study in mature lambs

At the same time as Experiment 4 was being carried out in Glasgow a similar vaccination experiment was carried out in collaboration with the Animal Diseases Research Association (ADRA) Edinburgh.

Experimental Design

The design of this experiment which is shown in Table 9 was essentially similar to Experiment 4 with the exception that the animals used were 10 month old Suffolk-Greyface cross lambs.

Results

Haematological Estimations

Packed cell volume percentages (PCVs)

The mean PCVs of all four groups are given in Figure 11 and the individual values presented in Appendix A. Neither of the two vaccinated groups (XI and XIII) showed any marked decrease in mean PCV during vaccination. By two to three weeks after challenge however, the mean PCVs of all groups decreased and with the exception of the Cambridge vaccinates fell below 30%

Parasitological Estimations

Faecal egg counts

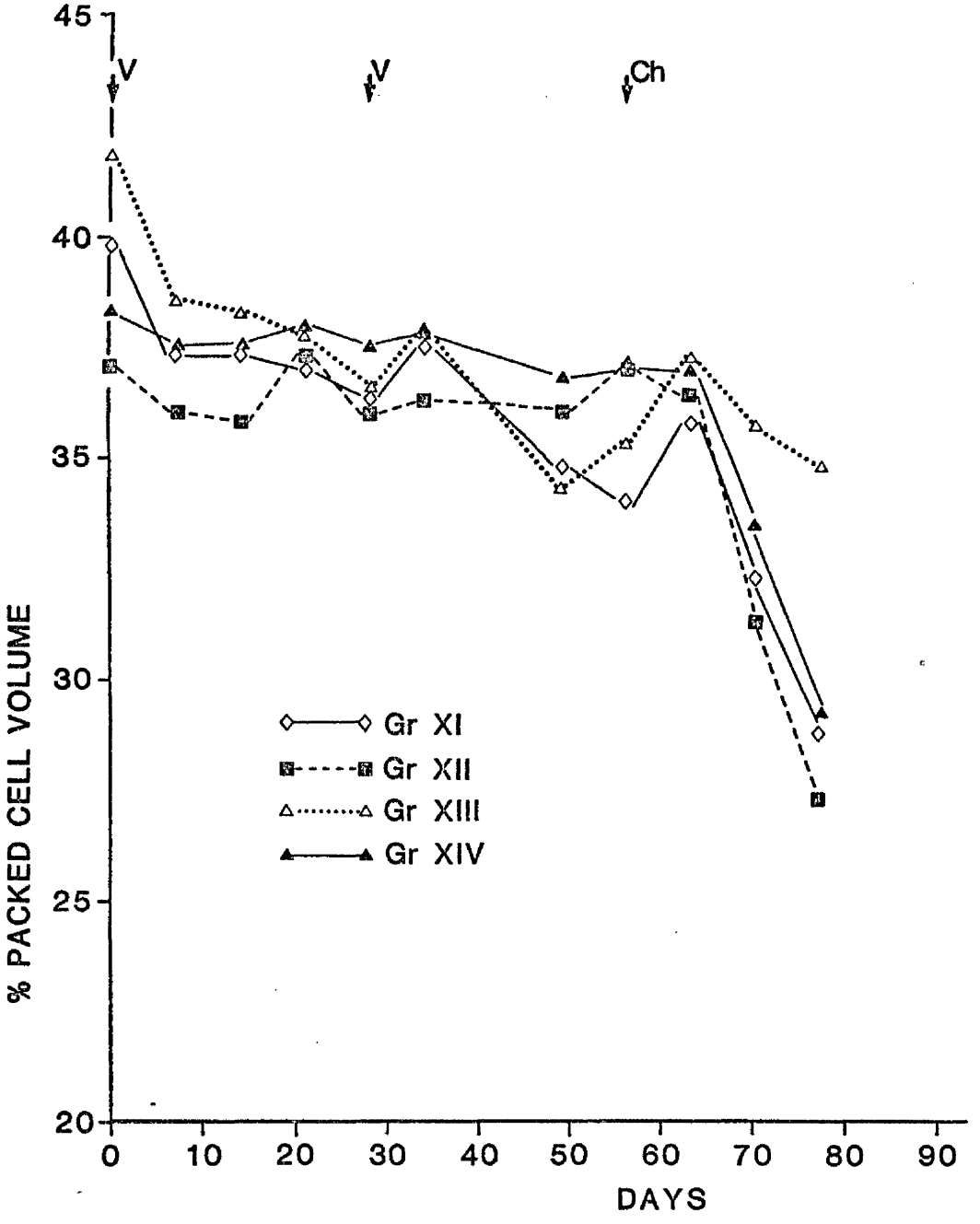
The mean faecal egg counts of all four groups are given in Figure 12 and the individual values are presented in Appendix A. Until 20 days after challenge infection faecal samples from all four groups of animals were consistently negative for nematode

Table 9

Design of Experiment 5

Group	No. of animals/ group	H. contortus strain	First vaccination Day 0	Second vaccination Day 28	Challenge infection Day 56	Necropsy Day 84
XI	4	Glasgow	10,000 L_3*	10,000 L_3*	10,000 L_3	K
XII	4	Glasgow	-	-	10,000 L_3	K
XIII	4	Cambridge	10,000 L_3*	10,000 L_3*	10,000 L_3	K
XIV	4	Cambridge	-	-	10,000 L_3	K

* 60 kr γ-irradiated larvae
K Killed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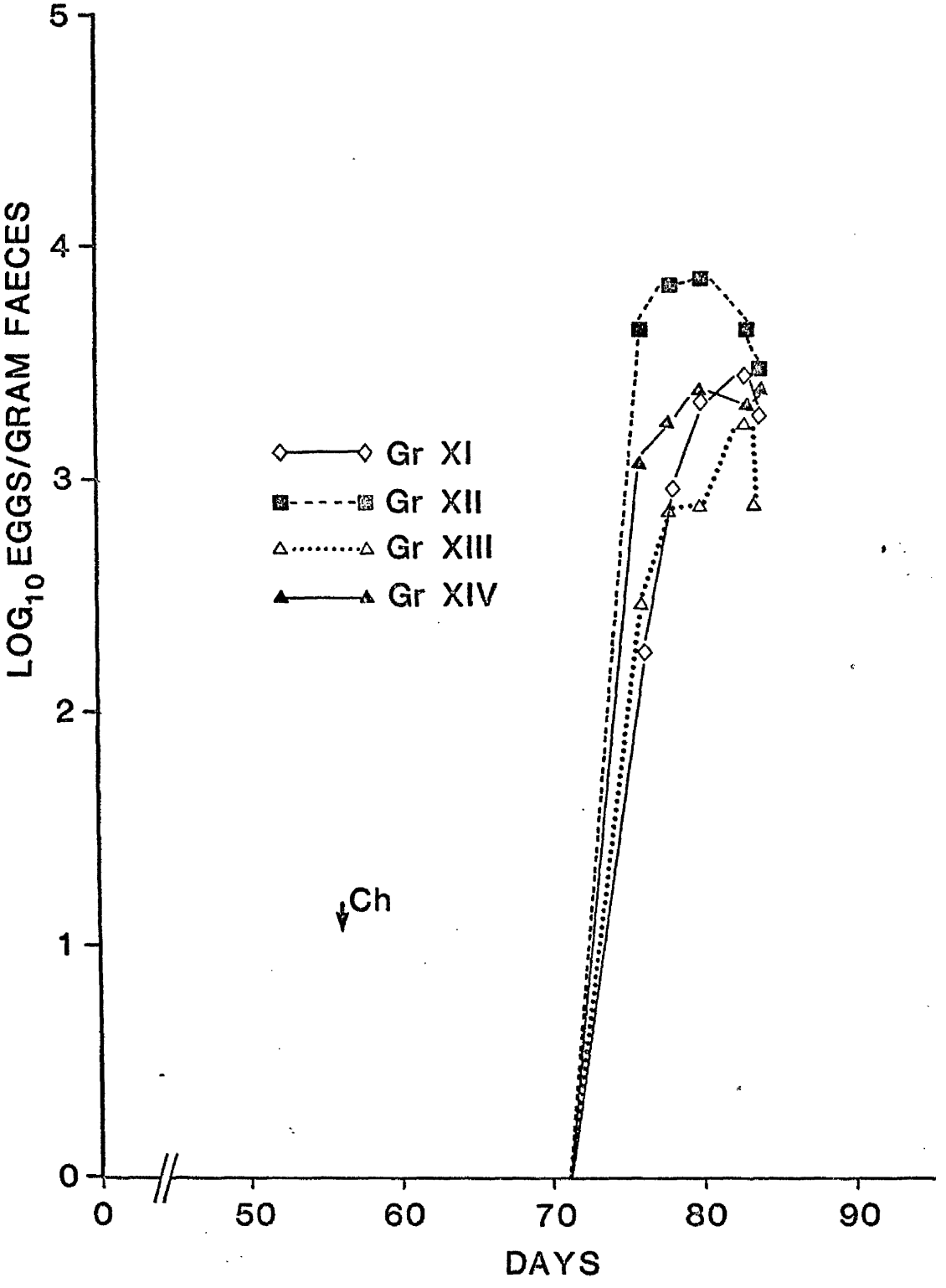
Gr XI
Gr XII
Gr XIII
Gr XIV

FIG 11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DAYS

FIG 12

eggs. Subsequently, positive counts were recorded in all four groups, the highest counts being recorded for the Glasgow controls.

Worm burdens

The mean total worm burdens of the four groups of animals are given in Table 10 and the individual counts with details of the various stages recovered are presented in Appendix A. Lambs from Groups XI and XII which were challenged with the Glasgow strain of H. contortus had extremely high worm burdens, one challenge control animal having 10684 worms at necropsy. A few immature stages were present in these two groups of lambs. The mean worm burdens of Groups XIII and XIV lambs i.e. infected with the Cambridge strain, were much lower being 1007 and 1454 respectively. Immature stages were recovered in both of these groups, particularly the vaccinated group.

Conclusions

It was obvious from the results obtained in this experiment in mature lambs that vaccination with both strains of larvae was unsuccessful in preventing the establishment of a significant number of challenge worms. In fact the results were similar to those obtained in the previous experiment using five month old lambs (Experiment 4). This is in contrast to all the earlier published reports.

The very high worm burdens recorded with the Glasgow strain of H. contortus gives some reason for unease and one might suspect that the larval doses were accidentally miscalculated. However, our collaborators at the ADRA were unable to find any evidence of this in retrospective checking.

In both vaccination experiments (Experiments 4 and 5) the field isolated Cambridge strain of H. contortus larvae showed signs of inhibition or delayed development. This was found to be more

Table 10

The mean (± SE) and range of worm burdens recovered at necropsy of the four groups of lambs

Group	No. of animals/ group	Vaccination/Challenge regimen			Worm burdens Mean	Range
XI	4	V_G	V_G	C_G	6401 ± 1235	2863 - 8597
XII	4			C_G	5999 ± 2177	945 - 10684
XIII	4	V_{Ca}	V_{Ca}	C_{Ca}	1007 ± 305	354 - 1816
XIV	4			C_{Ca}	1454 ± 285	872 - 2015

V - Vaccine
C - Challenge
G - Glasgow strain
Ca - Cambridge strain

marked in the older lambs and especially in the vaccinates. Since the inhibited development of the Cambridge isolate created further variables in these investigations it was decided not to use this strain in future studies.

DISCUSSION

The three experiments described in this chapter were undertaken to find out whether the Glasgow strain of *H. contortus* larvae had developed radiosensitivity due to regular passage under laboratory conditions and thereby had become less infective and less immunogenic. The radiosensitivity and immunogenicity of the Glasgow strain was compared with a completely unrelated and recently isolated field strain of *H. contortus* obtained from S.E. England (Cambridge strain).

Results obtained from the dose titration experiment (Experiment 3) indicated that when the % establishments of irradiated larvae were considered there was no evidence of over attenuation at 60 kr dose level as the mean worm burdens of the groups of lambs which received larvae irradiated at 40 kr and 60 kr were similar for both strains although the % establishment of the Cambridge strain was lower than that of the Glasgow strain for irradiated and normal larvae. The results obtained with the Glasgow strain were also similar to those obtained in the original studies by Jarrett and his colleagues (Jarrett *et al*, 1959; Urquhart *et al*, 1966a). Thus it was assumed that the Glasgow strain had not developed radiosensitivity in terms of worm establishment. Therefore, Experiments 4 and 5 were carried out using five-month-old and mature lambs to investigate the possibility that this strain may have lost its original immunogenicity.

It was obvious from the results obtained from these two experiments that double vaccination at monthly intervals was unsuccessful in preventing the establishment of a significant number of challenge worms administered a month after the second vaccination in both strains of larvae in mature and immature lambs. In earlier studies Urquhart *et al* (1966b) showed significant reductions in the challenge worm burdens of five-month-old lambs double vaccinated with *H. contortus*

larvae X - irradiated at 40 kr.

In both vaccination experiments (Experiment 4 and 5) the field isolated Cambridge strain showed signs of inhibition or delayed development. This inhibition was found to be more marked in older lambs especially in the vaccinates. In field studies in Cambridge, Connan (1971) demonstrated inhibition of H. contortus larvae during late summer and autumn. He also showed (Connan 1975) that the age of the host had an influence on inhibited development in that the larvae were less inclined to inhibition in very young animals. To avoid further variables in these vaccination studies due to inhibition of larval development the Cambridge strain was not used in the following studies.

SUMMARY

The findings of two studies using irradiated larvae are reported in this chapter. The first of these was a titration experiment to examine the attenuating effect of 40 kr and 60 kr γ - irradiation on infective larvae.

The results when compared with those of the previous experiments, in terms of sterile female worms and sex ratio, showed no evidence that the strain maintained in this laboratory had become more radio-sensitive.

The second study involved two experiments designed to assess the protection derived from two doses of larvae irradiated at 60 kr. Both of these experiments were unsuccessful when compared with previously published reports and confirmed the earlier finding that the 60 kr irradiated larval vaccine failed to confer protection against subsequent challenge.

CHAPTER III

VACCINATION STUDIES USING NORMAL AND ATTENUATED *HAEMONCHUS CONTORTUS* LARVAE.

Experiment 6: The immunising effect of two doses of normal *H. contortus* larvae against subsequent challenge

Introduction

Since previous experiments had indicated that irradiated *H. contortus* larvae of the laboratory strain maintained in Glasgow seemed to have lost their immunising properties a small experiment was carried out to examine the immunogenicity of this strain of larvae. This study involved two sensitising infections and a subsequent challenge infection of a group of lambs with normal larvae.

Experimental Design

The design of the experiment is given in Table 11. A total of six parasite naive lambs were used and these were twelve months old at the beginning of the experiment. The lambs were divided into two equal groups based on weight, sex and haemoglobin type. At the beginning of the experiment lambs from Group I each received a primary infection consisting of 10,000 *H. contortus* larvae orally. Four weeks after the first infection these lambs were given a second infection consisting of 10,000 L_3. Group II lambs remained uninfected during this period. Three weeks after the second infection lambs from Groups I and II each received a dose of the anthelmintic levamisole followed one week later by a dose of 10,000 normal *H. contortus* L_3. Four weeks after challenge all the lambs were necropsied.

Results

Haematological Estimations

Packed cell volumes (PCVs)

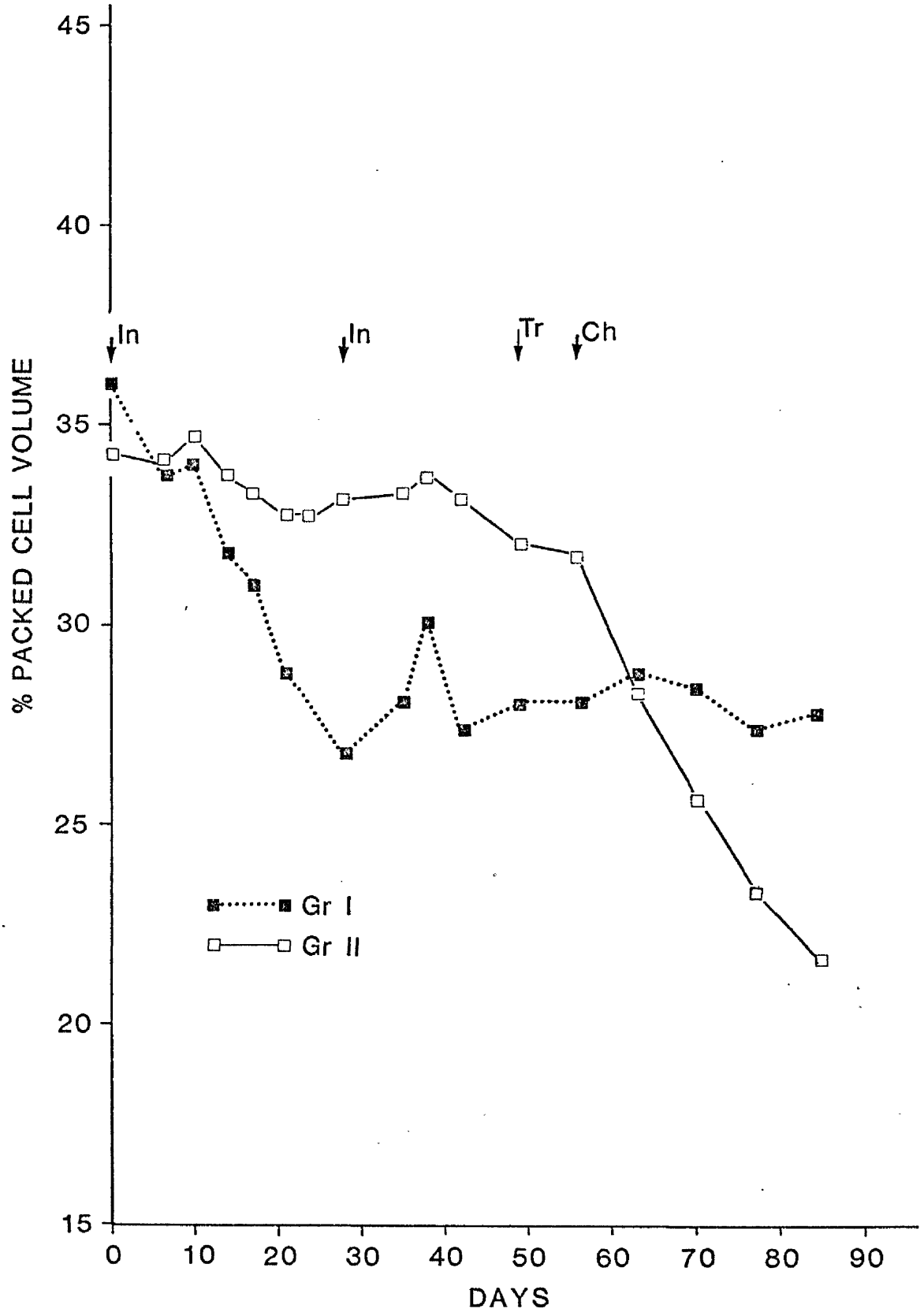
The mean PCVs of the two groups are shown in Figure 13 and the individual values are presented in Appendix A. The mean PCV of the Group I lambs decreased from 36% to 27% after the first infection but subsequently remained between 27-30% till the end

Table 11

Design of Experiment 6

Group	No. of animals/ group	First infection Day 0	Second infection Day 28	Anthelmintic treatment Day 49	Challenge infection Day 56	Necropsy Day 84
I	3	10,000 L_3	10,000 L_3	LEV	10,000 L_3	K
II	3	-	-	LEV	10,000 L_3	K

K Killed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DAYS

FIG 13

of the experiment. The mean PCV of the challenge controls (Group II) showed a marked decrease falling from around 32% to their lowest mean value of under 22% (21.7%) four weeks after the infection.

Parasitological Estimations

Faecal egg counts

The mean faecal egg counts of the two groups from the beginning of the experiment are given in Figure 14 and the individual values are presented in Appendix A. Twenty-one days after the first infection eggs appeared in the faeces of the Group I lambs and their weekly samples remained positive till anthelmintic treatment on day 49. Twenty-one days after challenge all of the lambs had positive faecal egg counts and peak values were reached four weeks after challenge in both groups. The egg counts of the challenge control lambs in Group II were considerably higher than those of the previously infected Group I lambs.

Worm burdens

The individual and mean worm burdens of the lambs in Groups I and II are given in Table 12 and details of the individual worm burdens are presented in Appendix A. The mean worm burdens of Groups I and II were 1983 and 4217 respectively. Although statistically there was no significant difference between the group worm burdens ($P = 0.1$) two of the three preinfected lambs had low worm burdens of 200 and 800 worms.

Conclusions

This small experiment was undertaken to establish whether two doses of normal larvae of Glasgow strain given at an interval of one month apart would stimulate resistance to a challenge infection given one month after the last infection.

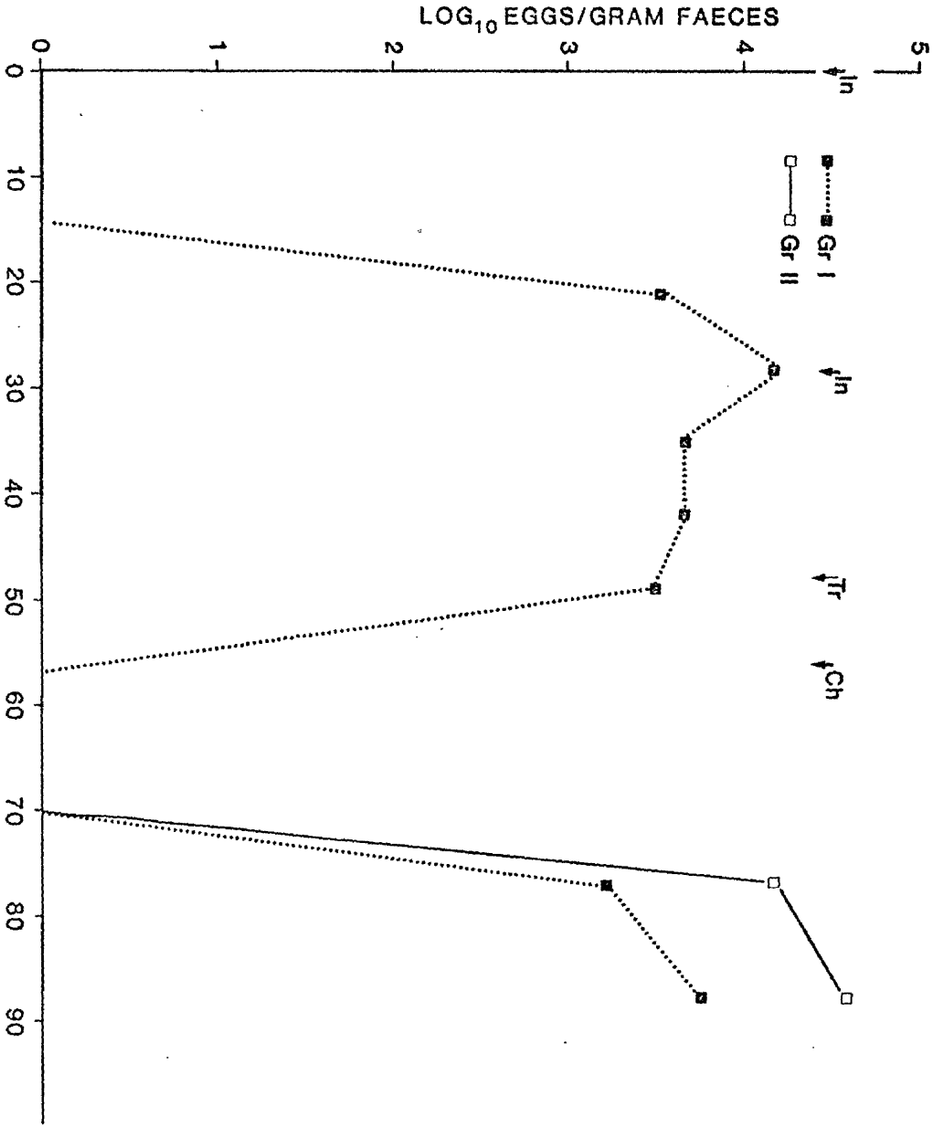
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FIG 14

Table 12

The individual and mean (± SE) worm burdens recovered at necropsy of the two groups of lambs

Group	No. of animals/ group	Infection/Treatment/Challenge regimen				Worm burdens
I	3	I	I	LEV	C	4950 200 800 1983 ± 1493
II	3			LEV	C	1800 5600 5250 4217 ± 1213

I Infection
C Challenge

There was no significant difference between the worm burdens of both groups.

Unfortunately, because of the small size of the groups i.e. three sheep in each, the results were somewhat equivocal. The mean worm burdens of 1983 to 4217 in the immunised and control groups respectively, were not significantly different as determined by the Mann-Whitney test. Nevertheless this does represent a 53% reduction in the mean worm burden of the immunised group; also two of the three immunised sheep had low worm burdens of 200 and 800.

A previous report on the degree of immunity stimulated by two doses of normal larvae is that of Manton et al (1962) who found that a group of eight mature lambs immunised with two doses of 4,500 H. contortus larvae at an interval of one month and challenged 30 days later with five daily doses of 3,000 larvae had a mean worm burden of 23 worms compared to 6023 in the control group - a reduction in worm burden of 99.6%. Their strain of larvae was a derivative of the Glasgow strain although their experiment was carried out 20 years ago.

In their experiment an anthelmintic was not used but it seems unlikely that the use of levamisole in the current experiment was responsible for the apparent absence of immunity since Smith and Christie (1979) have reported that levamisole did not interfere with the immune response stimulated by vaccination with irradiated H. contortus larvae when a single dose of anthelmintic was given three weeks after the immunising schedule (i.e. one week before challenge).

One might perhaps tentatively conclude that this experiment has demonstrated a relative lack of immunogenicity in the larvae used.

Experiment 7 A vaccination study using two laboratory strains of H. contortus and two sources of irradiation

Introduction

To further explore the apparent loss of immunogenicity of the Glasgow strain of H. contortus an experiment was designed where the radiation dose was reduced from 60 kr to 40 kr and another laboratory maintained strain of H. contortus was included for comparison. In addition, both γ - radiation and X - radiation were used as both types of irradiation had proved successful in earlier vaccination studies, recent failures being confined to experiments using the ^{60}Co source.

Experimental Design

The design of the experiment is given in Table 13. A total of 24, nine month old lambs were used. The lambs were divided into six groups of four based on weight, sex and haemoglobin type. At the beginning of the experiment lambs from Groups III and IV each received 10,000 Glasgow strain H. contortus larvae irradiated at 40 kr by γ - rays and X - rays respectively. At the same time lambs from Groups VI and VII each received a dose of 10,000 H. contortus larvae obtained from the laboratories of Merck Sharp and Dohme (MSD) irradiated at 40 kr by γ - rays and X - rays respectively. This vaccination procedure was repeated four weeks later. Groups V and VIII lambs remained uninfected during this period. Four weeks after the second vaccination the lambs from Groups III, IV and V each received 10,000 normal Glasgow strain H. contortus larvae while lambs from Groups VI, VII and VIII each received 10,000 normal MSD strain H. contortus larvae. Four weeks after the challenge infection all animals were necropsied.

Table 13

Design of Experiment 7

Group	No. of animals/ group	*H. contortus* strain	Irradiation dose and source	First vaccination Day 0	Second vaccination Day 28	Challenge infection Day 56	Necropsy Day 84
III	4	Glas	40 kr γ - ray	10,000 L_3*	10,000 L_3*	10,000 L_3	K
IV	4	Glas	40 kr X - ray	10,000 L_3*	10,000 L_3*	10,000 L_3	K
V	4**	Glas	-	-	-	10,000 L_3	K
VI	4	MSD	40 kr γ - ray	10,000 L_3*	10,000 L_3*	10,000 L_3	K
VII	4	MSD	40 kr X - ray	10,000 L_3*	10,000 L_3*	10,000 L_3	K
VIII	4	MSD	-	-	-	10,000 L_3	K

** One animal died during the course of the experiment
* Irradiated larvae (40 kr)
Glas Glasgow
MSD Merck Sharp and Dohme
K Killed

Results

Haematological Estimations

Packed cell volumes (PCVs)

The mean PCVs of all six groups are shown in Figure 15 and the individual values are presented in Appendix A. There was a gradual decrease in the PCVs of all four vaccinated groups during the course of vaccination. Four weeks after challenge the mean PCVs of the Glasgow vaccinates, Groups III and IV showed some recovery and were higher than those of the MSD vaccinated lambs in Groups VI and VII, which were showing a slight fall in PCVs at this time. The PCVs of both challenge control groups (V and VIII) showed a similar decrease soon after challenge reaching mean values of between 21% and 23% by four weeks.

Parasitological Estimations

Faecal egg counts

Throughout the vaccination period faecal samples from the lambs in all four vaccinated groups (III, IV, VI and VII) were negative for parasite eggs and after challenge the Glasgow vaccinates in Groups III and IV remained negative.

The mean faecal egg counts of the MSD vaccinates and the challenge controls are given in Figure 16 with individual values in Appendix A. Three weeks after challenge eggs appeared in the faeces of lambs from all four groups with the exception of two lambs from Group VI which had negative counts after challenge. Peak values were recorded one week later.

Worm burdens

The mean worm burdens of the lambs from all six groups are given in Table 14 and the individual counts with the details of the various stages recovered are presented in Appendix A. Lambs vaccinated with the Glasgow strain i.e. Groups III and IV had significantly lower worm burdens than their controls in Group V,

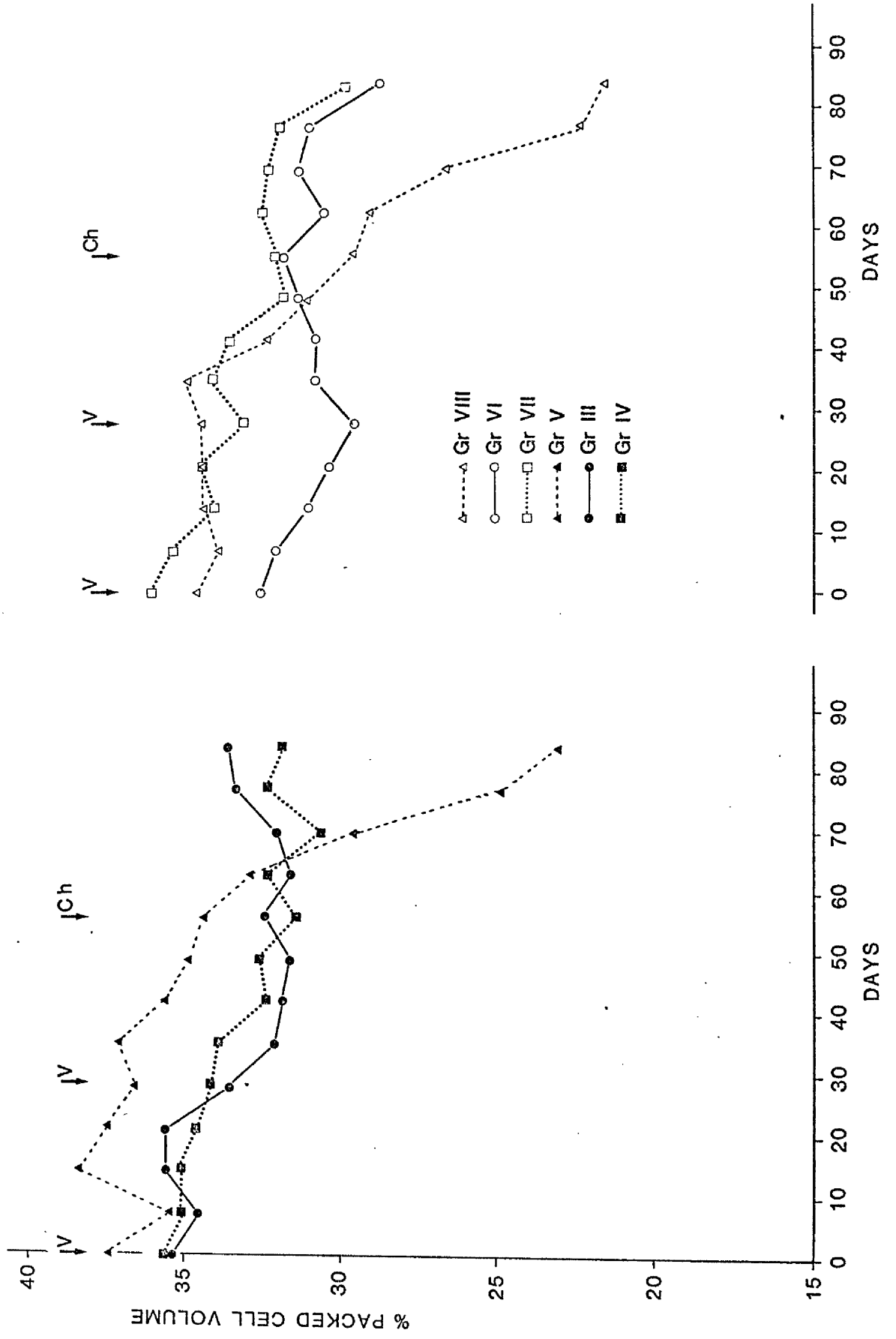
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FIG 15

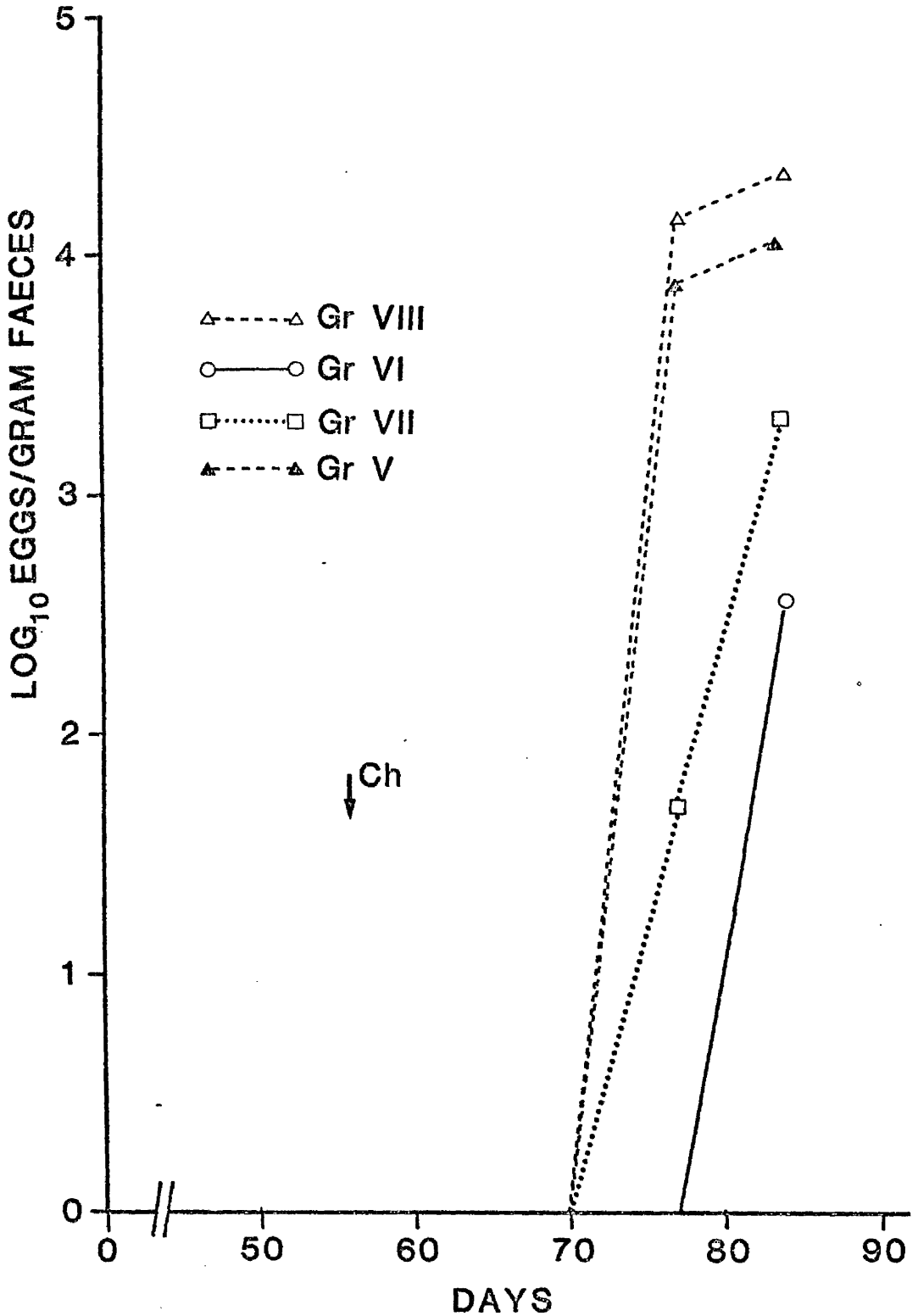
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FIG 16

Table 14

The mean (± SE) and range of worm burdens recovered at necropsy of the six groups of lambs

Group	No. of animals/ group	Vaccination/Challenge regimen			Worm burdens	
					Mean	Range
III	4	V_G	V_G	C_G	75 ± 14	50 - 100
IV	4	$V_{G\ X}$	$V_{G\ X}$	C_G	263 ± 133	50 - 600
V	4*			C_G	4833 ± 891	3750 - 6600
VI	4	V_{MSD}	V_{MSD}	C_{MSD}	1838 ± 1177	0 - 5200
VII	4	$V_{MSD\ X}$	$V_{MSD\ X}$	C_{MSD}	3250 ± 1218	400 - 5900
VIII	4			C_{MSD}	4075 ± 401	2950 - 4850

* One animal died during the course of the experiment
G Glasgow strain
MSD Merck Sharp and Dohme strain
V Vaccine
C Challenge
$\gamma\ \gamma$ - irradiation
X X - irradiation

while the worm burdens of the MSD strain vaccinates in Groups VI and VII were not significantly different from those of their controls in Group VIII. No worms were recovered from one lamb from Group VI. Whereas the majority of the worms recovered from Groups III and IV were sterile females half of those recovered from Groups VI and VII were normal adult worms and the other half sterile females.

Conclusions

In this experiment, the results of vaccination with the Glasgow strain of H. contortus larvae were, for the first time in this study, similar to those previously reported in that a reduction in the mean worm burdens of the order of 98.5% and 94.5% was achieved after challenge in the two vaccinated groups compared to the control group. In fact, the degree of protection was nearer 100% in both vaccinated groups since the majority of the worms recovered in these groups were sterile females, almost certainly a residue of the vaccination regimen.

The degree of protection was also reflected in the relative changes in PCVs and in the faecal egg counts.

The only apparent conclusion of this experiment is that successful immunisation with this repeatedly passaged strain of H. contortus now depends on reducing the irradiation dose to 40 kr rather than the 60 kr ^{60}Co irradiation used in some previous experiments. Precisely why this should be so is obscure. The irradiation titration experiment reported in the previous chapter (Experiment 3) showed that the numbers of irradiated worms, primarily sterile females, were similar whether a 40 kr or 60 kr irradiation dose was used and one might have assumed that the worm burdens were the primary correlate of immunogenicity.

In comparison, vaccination with the MSD strain of H. contortus failed to confer protection against challenge with the MSD strain at least as measured by the Mann-Whitney U test, although the worm burdens of the vaccinated groups were reduced by 55% and 20% with the γ - irradiation and X - irradiation procedures respectively. The lack of protection was also evident from the results of PCVs and the faecal worm egg counts of the vaccinated animals.

Experiment 8 A study of the immunogenicity of two laboratory strains of H. contortus

Introduction

This experiment was carried out to confirm the result obtained in the previous experiment that is when a 40 kr irradiation dose was used a vaccine prepared with the Glasgow strain of H. contortus larvae produced a strong immunity against subsequent challenge with normal Glasgow larvae, whereas a similar vaccine of irradiated MSD strain larvae did not protect against challenge with normal MSD larvae. As there was no evidence of any difference between the vaccines produced by γ - rays and X - rays, γ - irradiation was used in this experiment but the Glasgow strain larvae was irradiated at two levels namely 40 kr and 60 kr while MSD larvae were irradiated at 40 kr only.

Experimental Design

The design of the experiment is given in Table 15. A total of 25 worm-free eight-month old lambs were used. The lambs were divided into five equal groups based on weight, sex and haemoglobin type. At the beginning of the experiment lambs from Group IX and X each received 10,000 Glasgow strain H. contortus larvae γ - irradiated at 40 kr and 60 kr respectively. At the same time Group XII lambs each received 10,000 MSD strain H. contortus larvae γ- irradiated at 40 kr. Four weeks later this vaccination procedure was repeated. Groups XI and XIII lambs remained uninfected during this period. Four weeks after the second vaccination lambs from Groups IX, X and XI each received 10,000 normal Glasgow strain H. contortus larvae while lambs from Groups XII and XIII each received 10,000 normal MSD larvae. Four weeks after the challenge infection all the lambs were necropsied.

Table 15

Design of Experiment 8

Group	No. of animals/ group	*H. contortus* strain	Dose of irradiation (γ - ray)	First vaccination Day 0	Second vaccination Day 28	Challenge infection Day 56	Necropsy Day 84
IX	5	Glas	40 kr	10,000 L_3*	10,000 L_3*	10,000 L_3	K
X	5	Glas	60 kr	10,000 L_3*	10,000 L_3*	10,000 L_3	K
XI	5	Glas	-	-	-	10,000 L_3	K
XII	5**	MSD	40 kr	10,000 L_3*	10,000 L_3*	10,000 L_3	K
XIII	5	MSD	-	-	-	10,000 L_3	K

** Two animals died during the course of the experiment
Glas Glasgow
MSD Merck Sharp and Dohme
* irradiated larvae
K Killed

Results

Two lambs from Group XII died during the course of the experiment due to severe pneumonia.

Haematological Estimations

Packed cell volumes (PCVs)

The mean PCVs of all five groups are given in Figure 17 and the individual values are presented in Appendix A. The PCVs of the vaccinated groups decreased but showed a pattern similar to that of the control lambs during the period of vaccination. After challenge the PCVs of Groups IX, X and XI showed further decreases: the PCVs of the MSD vaccinates in Group XII however, remained at the same level whereas their challenge controls in Group XIII showed a marked fall in PCV, a value of 27% being recorded four weeks after challenge.

Parasitological Estimations

Faecal egg counts

Throughout the vaccination period faecal samples from lambs in all three vaccinated groups proved negative for parasite eggs. The mean faecal egg counts of all five groups after challenge are given in Figure 18 and the individual values are presented in Appendix A. Lambs from Groups XI, XII and XIII were positive for H. contortus eggs from day 16 after infection onwards. Except for one lamb from Group IX and two lambs from Group X which gave low egg counts during the fourth week after challenge the Glasgow vaccinated lambs remained negative for eggs. High egg counts were recorded from the challenge control Groups XI and XIII and peak values were reached in these groups four weeks after the challenge infection.

Worm burdens

The mean worm burdens of lambs from all five groups are

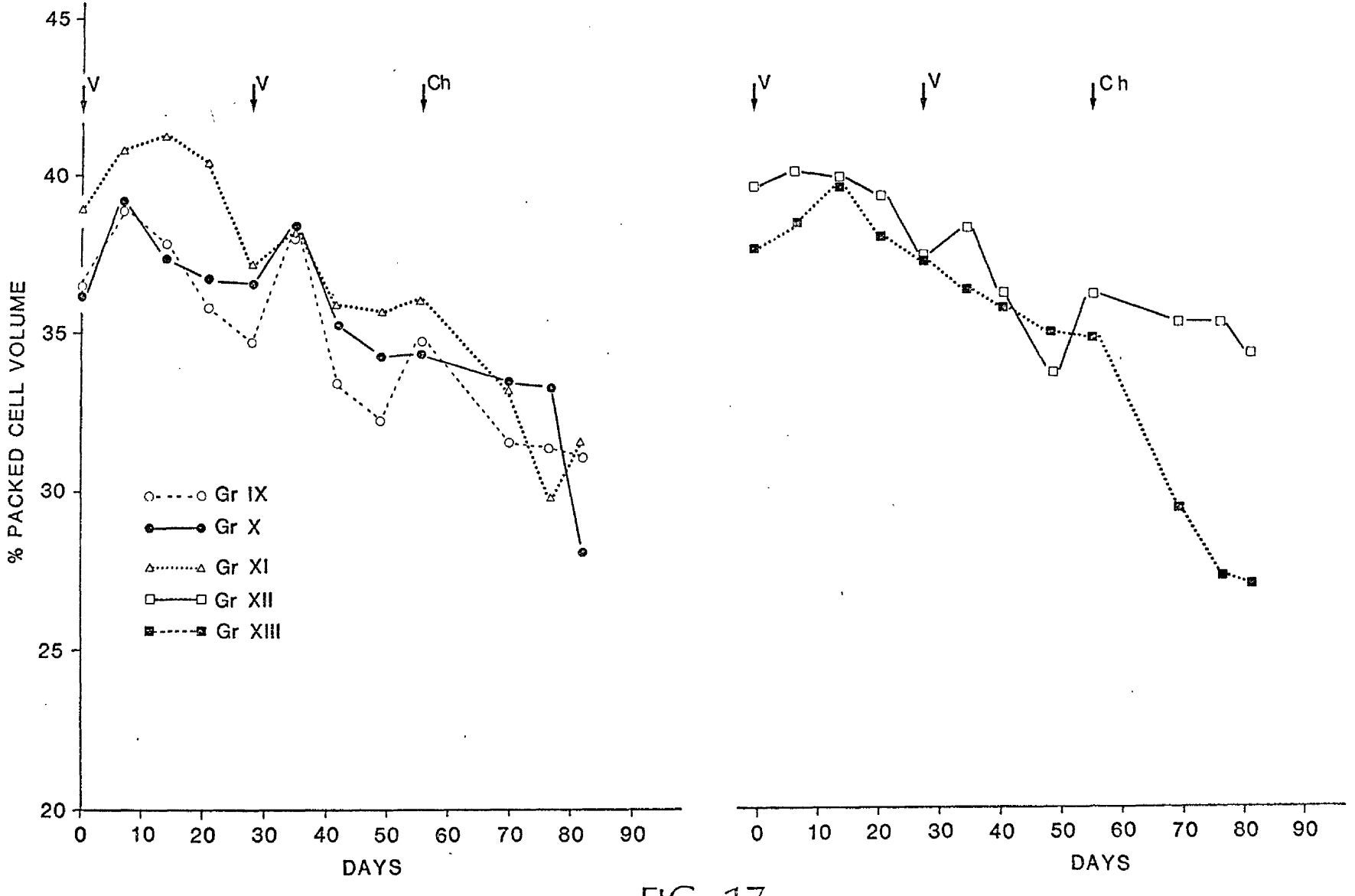
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FIG 17

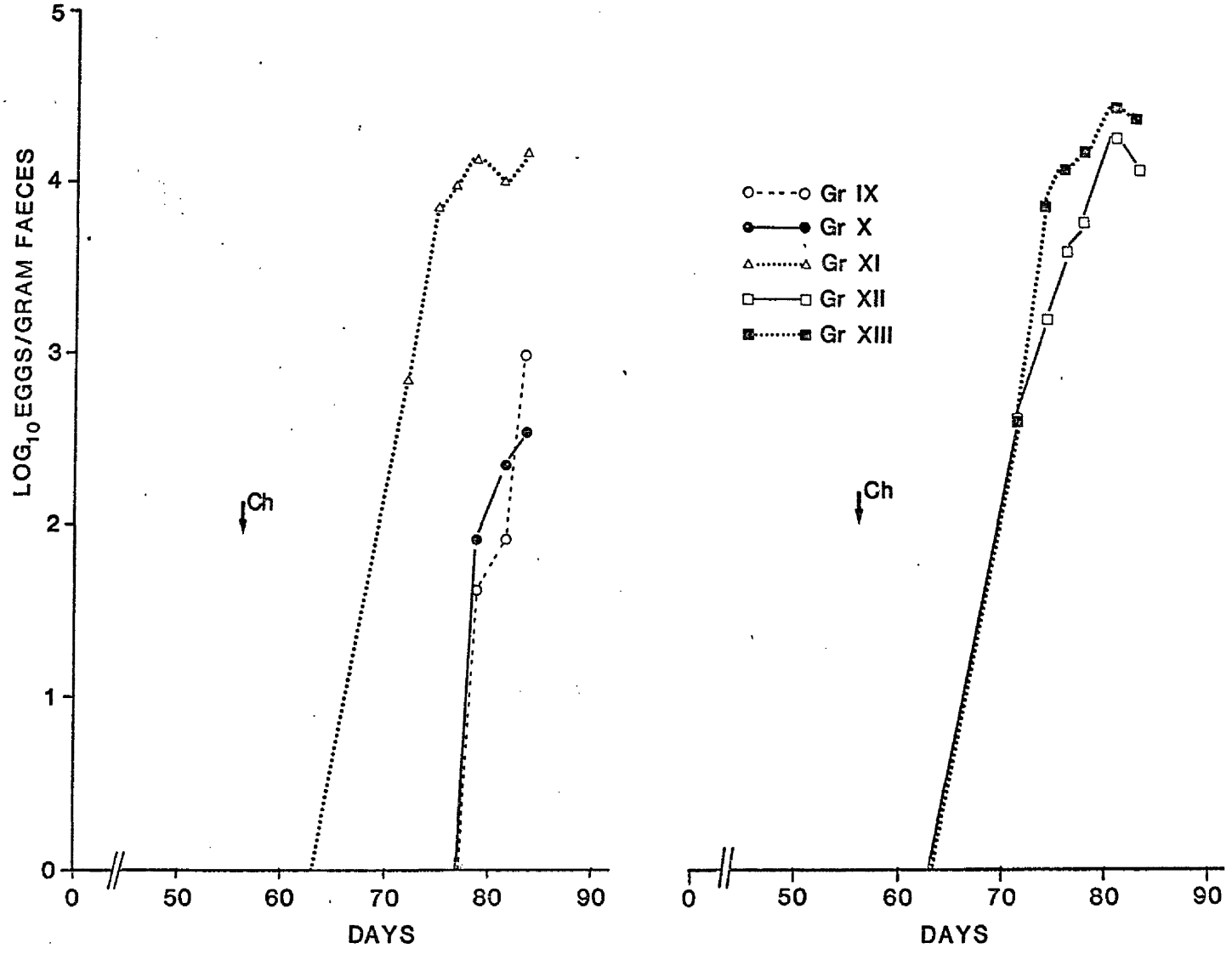
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FIG 18

given in Table 16 and the individual worm burdens are presented in Appendix A. Both the challenge control groups (XI and XIII) had high worm burdens with means of over 2000. Lambs from the Glasgow vaccinated Groups IX and X had worm burdens significantly lower than their controls in Group XI. Group IX lambs had the lowest worm burdens and most of these were sterile females. Approximately 30% of the worms recovered from the three lambs in the MSD vaccinated Group XII were sterile females. There was no significant difference between the worm burdens of Groups XII and XIII ($p = 0.07$).

Conclusions

The objective of this experiment was to confirm that successful immunisation, at least with the Glasgow strain of H. contortus larvae, depended on irradiation at 40 kr rather than at 60 kr, a conclusion indicated by the previous experiment.

Rather surprisingly both vaccination regimens were successful with mean adult worm burdens of 290 and 610 in the 40 kr and 60 kr vaccinated groups respectively compared to 2550 in the control group. The faecal egg counts of these three groups reflected the worm burdens and seven out of 10 immunised lambs had negative egg counts after challenge infection.

Immunisation with the MSD strain did not confer a significant degree of protection although the group mean worm burden was reduced by 38%, a result similar to that obtained in the previous experiment (Experiment 7).

Table 16

The mean (± SE) and range of worm burdens recovered at necropsy of the five groups of lambs.

Group	No. of animals/ group	Vaccination/Challenge regimen			Worm burdens Mean	Range
IX	5	$V_{G\ 40}$	$V_{G\ 40}$	C_G	200 ± 107	0 - 550
X	5	$V_{G\ 60}$	$V_{G\ 60}$	C_G	610 ± 324	0 - 1700
XI	5			C_G	2550 ± 282	1750 - 3200
XII	5*	$V_{MSD\ 40}$	$V_{MSD\ 40}$	C_{MSD}	1250 ± 558	150 - 1950
XIII	5			C_{MSD}	2010 ± 130	1700 - 2400

* Two lambs died during the course of the experiment
V Vaccine
C Challenge
G Glasgow
MSD Merck Sharp and Dohme
40/60 γ - irradiation dose

DISCUSSION

The series of experiments described in this section of the thesis was not that originally planned to investigate the effect of anthelmintics on the immune response of sheep to infection with H. contortus. This was due to the entirely unforeseen result of the first experiment when almost identical mean worm burdens of 2480 and 2733 were recovered from the sheep vaccinated with two doses of irradiated larvae and from the control sheep respectively. Previously published reports using an identical regimen of vaccination indicated that the worm burdens of the vaccinated sheep should have been reduced by around 97% (Benitez-Usher et al, 1977; Smith and Christie, 1978). Subsequent experiments were therefore devoted to an attempt to establish the cause of the vaccination failure.

On consideration, it seemed that there were three possibilities which might be responsible. These were:

a) a technical error in the preparation of the vaccine

b) a loss of immunogenicity in our strain of H. contortus which has been passaged for years in this laboratory

c) increased radiosensitivity of the H. contortus larvae leading to over-attentuation and loss of infectivity and immunogenicity. This also could be associated with continuous passage.

In a retrospective investigation of the first possibility, which included recalibration of the cobalt source, nothing was found which might indicate error. The likelihood of this was further discounted when Dr. W.D. Smith of the Animal Diseases Research Association, Edinburgh informed us (personal communication) that he also had two recent experiences of vaccine failure despite having previously conducted several successful experiments (Smith and Christie, 1978; 1979; Smith and Angus, 1980). Since he maintained

his own culture of H. contortus, used his own technical staff and a separate cobalt source it seemed likely that some other factor was involved.

Since Dr. W.D. Smith's strain of H. contortus was derived from the Glasgow strain some 10 years previously and had been passaged at approximately the same rate ever since, it was considered that both strains might have lost their immunogenicity. Information on this aspect was obtained from Experiments 2 and 6. In Experiment 2 a group of five sheep was double-vaccinated with irradiated larvae followed by an infection with normal larvae before challenge 28 days later. The immunity produced was not significant as assessed by the Mann-Whitney test although there was a reduction of 59% in the mean worm burden of the vaccinated group compared to the controls.

In the second experiment (Experiment 6) each of three sheep were given two infections of 10,000 normal larvae a month apart, treated with levamisole 21 days after the second infection and seven days later were challenged with 10,000 larvae. At necropsy the mean worm burdens were not significantly different from the control group, although the immunised group had a reduction of 53% in their mean worm burden.

When one compares these results with similar highly successful vaccination experiments conducted along the same lines although with slightly different regimens (Manton et al, 1962; Smith and Christie, 1979) it seems not unreasonable to suspect that the current strain lacks its former immunogenicity. This is reinforced by the fact that the two groups of workers quoted above had both obtained their strain of H. contortus from Glasgow some years ago.

In an attempt to investigate this aspect further a new strain of H. contortus was studied. This was obtained from Dr.

R.M. Connan of the University of Cambridge and was a comparatively recent and completely different isolate from the Glasgow strain. The Cambridge strain was compared to the Glasgow strain in two experiments (Experiments 4 and 5) in which larvae irradiated with 60 kr from a cobalt source were used in two immunising infections. Both experiments showed that the Cambridge strain was as ineffectual as the Glasgow strain in stimulating protection against challenge. This negative result is unfortunately of little value in assessing a possible loss of immunogenicity in the Glasgow strain.

The third possibility, that the current strain of Glasgow H. contortus had become more radiosensitive was then examined.

As a first step (Experiment 3) two batches of larvae of the Glasgow strain were irradiated in a cobalt source at 40 kr and 60 kr respectively. These were given in doses of 10,000 larvae to groups of three sheep and at necropsy 26 days later the worm burdens were counted and compared with those of control sheep given 10,000 normal larvae. The results showed that the worm burdens of the sheep which received larvae irradiated at 40 kr and 60 kr respectively were reduced by 68% and 73%. In an earlier experiment with the same strain of larvae, Jarrett et al (1959) found that the worm burdens of sheep infected with larvae irradiated at 40 and 60 kr were both reduced by 87%. The only difference in their experiment was that the irradiation was conducted with X-rays. This experiment therefore provides no evidence at all which might indicate that the larvae had become more radiosensitive, although it could be argued that infectivity is not necessarily correlated with immunogenicity.

Looking back over the historical background of immunisation against H. contortus with irradiated larvae it is apparent that whereas the earlier experiment utilised 40 kr irradiation dose

delivered from an X - ray source, more recent experiments from the time of Benitez-Usher and his colleagues (1977) used 60 kr γ - rays from a cobalt source. The reasons for this change are not clear but perhaps depended on two factors. First, the ready availability of the cobalt source compared to the type of X - ray machine required which is only found in radiotherapy units and is generally only available for other uses after normal working hours. Secondly, the fact that 60 kr rather than 40 kr is now used may be due to the fact that results, at least as good as those obtained with 40 kr have been reported with both X - rays (Jarrett et al, 1959) and γ - rays (Benitez-Usher et al, 1977; Smith and Christie, 1978).

In view of the impasse with the irradiation dose of 60 kr in this series of experiments, it was decided to conduct an experiment (Experiment 7) using an irradiation dose of 40 kr. Two groups of four sheep were vaccinated, one with larvae irradiated in a cobalt source and the other with larvae irradiated by X - rays. When the sheep were necropsied after challenge the mean worm burdens were found to be 75 and 263 respectively while a mean of 4883 worms were found in the control group i.e. reductions of 98.5% and 95% in the two vaccinated groups.

Since this was the first experiment of the series to produce a high degree of protection, comparable to those reported by other workers and also the first to use an irradiation dose of 40 kr, it was tentatively concluded that the factors which determine immune genicity had in fact become more radiosensitive, perhaps through the effects of continued rapid passage.

A final experiment to confirm this hypothesis was then undertaken in which two groups, each of five sheep, were double-vaccinated with larvae irradiated with γ - rays at 40 kr and 60 kr

respectively before challenge with 10,000 larvae. The results showed that irradiation at 40 kr again worked very successfully with a 92% reduction in the mean worm burden compared to the controls. However, the 60 kr level of irradiation was apparently not quite so successful with a 76% reduction in worm burden although this was not significantly different from that of the 40 kr group.

The reason why irradiation of larvae at 60 kr was relatively effective in this last experiment and yet failed to immunise in identical experiments carried out previously in this series must be conjectural. Interpretation is also complicated by the inevitable variability in worm burdens of H. contortus and the relatively small numbers of sheep able to be used in each group.

Within these limitations, if one summarises the results of the four experiments solely concerned with double-vaccination with larvae irradiated at 60 kr and subsequent challenge, the degree of protection was as follows:-

Experiment 1. A 9% reduction in worm burden; however, if the unusually high numbers of sterile females, presumably derived from the double-vaccination procedure are excluded, this could be taken as 48%.

Experiment 4. A 39% reduction in worm burden; this experiment was, of course, conducted with five-month-old lambs in which a degree of protection of the order of 64% was previously reported (Urquhart et al, 1966 b).

Experiment 5: No reduction in worm burden.

Experiment 8. A 76% reduction in worm burden.

In comparison, the three vaccination studies (Experiments 7 and 8) carried out with 40 kr, using either γ - rays or X - rays, gave a degree of protection of 98.5%, 95% and 92% respectively.

As a working conclusion it therefore seems that with the current state of the Glasgow strain of H. contortus it is now necessary

to use 40 kr rather than 60 kr of irradiation, from either a γ - ray or an X - ray source, to achieve consistently the maximum level of protection against challenge. This is apparently not due to an increased degree of radiosensitivity but rather to the fact that the strain seems to have lost some of its innate immunogenicity (Experiments 2 and 6). Why this should be 'restored' by irradiation at 40 kr rather than 60 kr is not clear.

It is perhaps possible that irradiation at 60 kr has in fact always been a dangerously high level for good immunogenicity and that, more by good luck than good judgement, successful results were achieved in the past. This variability in results might also be associated with the physiological state and innate infectivity of various batches of larvae before being subjected to irradiation.

The results of this study clearly indicates the necessity for more work on the mechanisms underlying the immunogenicity of irradiated larvae which apart from the studies conducted by Mulligan and his colleagues (Jennings, Mulligan and Urquhart, 1963; Prochezka and Mulligan, 1965; Kassai, Fitzpatrick and Mulligan, 1966; Fitzpatrick and Mulligan, 1967) have been neglected for many years. A better appreciation of this field might lead to a greatly increased understanding of the factors affecting immunogenicity.

A final point of interest was the fact that immunisation with the Merck Sharp and Dohme strain of larvae irradiated at 40 kr, either in an X - ray or cobalt source, was not particularly successful in stimulating immunity to challenge with the same strain. None of the mean worm burdens of the three immunised groups were significantly different from the control groups as measured by the Mann-Whitney test and in terms of reduction in mean worm burden was 55%, 20% and 38% respectively. Whether the MSD strain is basically less immunogenic than the Glasgow strain or whether this could

be 'restored' by irradiation at a level other than 40 kr is unknown, as is the degree of cross-protection which might be produced by immunisation with the Glasgow strain. However, this result provides further evidence of the importance of strain variation in helminth species (Mulligan et al, 1961; Poeschel and Todd, 1972; Kelly, Whitlock, Thompson, Hall, Martin, Le Jambre, 1978).

SUMMARY

In this chapter the results of three experiments are reported. In the first of these it was shown that two doses of normal Glasgow strain H. contortus larvae were unsuccessful in stimulating a significant degree of protection against subsequent challenge. The possibility that there had been a change in immunogenicity of the Glasgow strain of H. contortus since earlier successful experiments was discussed.

In the second experiment the immunising effect of the same strain of larvae irradiated at 40 kr was studied. For the first time a degree of protection similar to those reported in earlier studies was achieved i.e. in the order of 95% protection in terms of worm burdens, and X - irradiation and γ - irradiation were equally successful. Since this experiment raised the possibility that successful immunisation depended on irradiation at 40 kr instead of 60 kr the third experiment was primarily concerned with the comparison of these two irradiation doses. Successful results were again obtained with the 40 kr irradiated larval vaccine and for the first time in this series of experiments, a 60 kr irradiated larval vaccine also conferred protection. However, parallel experiments using another laboratory strain of H. contortus irradiated at 40 kr failed to immunise against challenge.

The significance of these results were discussed against the whole background of irradiated H. contortus larval vaccines.

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SECTION 2

NIPPOSTRONGYLUS BRASILIENSIS: SOME FACTORS INFLUENCING RESISTANCE TO REINFECTION IN RATS.

GENERAL INTRODUCTION AND REVIEW

In domestic animals anthelmintic treatment is accepted as essential in the treatment and control of infestations of gastro-intestinal helminths. Although the efficiency of such drugs is exhaustively documented there are, however, two aspects of their use which have received relatively little attention.

The first is the possible effect of the removal of an existing worm burden on the development of the immune response to subsequent infection with the same parasite(s). The second, that the development or maintenance of the immune response to the parasite might be modulated by the particular anthelmintic used.

The first aspect was studied in sheep in some detail by Dineen and his colleagues (Dineen and Wagland, 1966; Wagland and Dineen, 1967; Donald, Dineen and Adams, 1969) in experiments in which a series of Haemonchus contortus infections were terminated with the anthelmintic thiabendazole and this was followed by rechallenge of the sheep. The combined results of these experiments are somewhat difficult to interpret perhaps because in two of the three experiments the sheep were still immunologically immature to H. contortus infection during the early months of the experimental infection. It appeared, however, that strong resistance was associated with prolonged uninterrupted infection and that anthelmintic treatment was likely to interfere with the development of immunity. However, when the immunising infection produced high worm burdens i.e. circa 3000 worms little immunity developed to challenge unless the high worm burdens were removed by an anthelmintic eight days previously.

In 1970, Gibson, Parfitt and Everett (1970) demonstrated that under conditions of continuous daily infection monthly anthelmintic treatment did not interfere with the development of resistance to Trichostrongylus colubriformis in lambs. Subsequently similar

results were obtained with the sheep abomasal parasite Ostertagia circumcincta (Gibson and Parfitt, 1976).

In contrast, Boag and Thomas (1973) in an epidemiological study on gastro-intestinal nematodiasis in N.E. England concluded that dosing with the anthelmintic tetramisole at weaning may have interfered with the development of immunity since the highest faecal egg counts and the highest worm burdens (mainly species of Ostertagia, Trichostrongylus, Cooperia and Strongyloides) were found in the dosed lambs at necropsy in September compared to the similarly managed but undosed control animals.

Reverting to H. contortus infections in sheep, Benitez-Usher, Armour, Duncan, Urquhart and Gettinby (1977) observed that Blackface lambs vaccinated with irradiated larvae on two occasions at nine and 10 months of age failed to develop any immunity to subsequent challenge when thiabendazole was administered three weeks after each immunising dose; vaccinated controls, which received no anthelmintic were almost completely resistant to challenge. On the other hand Smith and Christie (1979) reported that after a similar vaccination regimen, treatment with levamisole on one occasion i.e. three weeks after the second dose of vaccine and one week before challenge did not affect protection. They suggested that the continued presence of vaccine worms from weeks three to seven of the immunising course might be crucial for the development of immunity. -Whether or not the anthelmintic used i.e. levamisole had an immunostimulant effect was not discussed.

More recently, Smith, Jackson and Jackson (1982) reported that sheep obtained some degree of protection to challenge with Ostertagia circumcincta larvae after immunisation with two doses of irradiated O. circumcincta larvae at monthly intervals but this protection was abolished when the immunising worm burdens were

removed by fenbendazole treatment one week before challenge.

The second aspect i.e. the possible immunomodulatory effect of anthelmintics seems to have received relatively little attention. However, levamisole hydrochloride, one of the most commonly used anthelmintics in veterinary medicine, is widely used in human medicine as an immunostimulant. In an extensive review of its role in this latter respect Symoens and Rosenthal (1977) stated that the accumulated evidence suggests that levamisole restores to normal the functions of phagocytes and T lymphocytes in immunologically compromised hosts. Therapeutic doses do not seem to increase the immune response above normal level. If this is indeed the case it might imply that levamisole, when used as an anthelmintic, might only act as an immunostimulant also if the host was immunosuppressed in respect of phagocytic ability and T cell function by virtue of the helminth infection or for some other reason.

In helminth infections there are a number of references to the immunomodulatory properties of levamisole as opposed to the previously quoted suggestion by Boag and Thomas (1973) that tetramisole perhaps suppressed the development of immunity to gastro-intestinal nematodes in sheep.

The first is by Liauw, Heymann and Barclay (1977) who showed that a delayed-type hypersensitivity reaction which could be elicited in the foot-pad of mice, seven days after infection with Nippostrongylus brasiliensis, by intradermal infection of adult worm antigen was significantly increased if levamisole was given at 10 mg/kg one or two days before challenge.

The second was the conclusion of Mitchell and Armour (1981) that sheep, primed with gastro-intestinal nematodes and then treated with levamisole on three occasions were resistant to a significant extent ($P < 0.05$) to challenge with 250 metacercariae of Fasciola

hepatica. Sheep receiving the nematode infection only or levamisole only had no increased resistance. Speculating on the role of levamisole in this experiment the authors state that "it seems reasonable to assume ..... levamisole acted by correcting the immunosuppression induced by the interaction of prior nematode and subsequent fluke infections".

Recently Oakley (1981) has reported that the protection stimulated by the administration of a single dose of viable Dictyocaulus viviparus larvae to calves was significantly increased when levamisole was administered to calves one day after the primary infection. This he attributed to the immunostimulant properties of the drug although an alternative explanation i.e. that the drug killed all of the larvae in an advantageous and immunogenic site in the intestinal wall or mesenteric lymph nodes perhaps deserves consideration.

Finally Duncombe, Bolin, Davis, Fagan and Kelly (1979) have shown that the anthelmintic efficacy of levamisole was not impaired against N. brasiliensis in rats maintained on an iron and protein deficient diet. In contrast the benzimidazole anthelmintics, mebendazole and fenbendazole were shown to be much less effective. The authors suggest that although the explanation might lie in the pharmacokinetics of these different drugs, in malnourished hosts it is possible that the levamisole acted as an immunostimulant as well as an anthelmintic.

The immunomodulatory properties of other anthelmintics have not been studied although it is perhaps relevant that both anti-inflammatory properties and the suppression of delayed hypersensitivity have been attributed to the drug thiabendazole (Campbell, 1971a,b; Hewlett, Hamid, Ruffier and Mahmoud, 1981).

Originally it was proposed to investigate these two aspects of anthelmintics i.e. the effect on the immune response of the

removal of the sensitising worm burden and the immunomodulatory effects, if any, of these drugs in immunologically mature lambs infected with H. contortus. It was hoped to develop the observations made by Benitez-Usher et al (1977). Unfortunately, because of the difficulties encountered with the irradiated larval vaccine of H. contortus described in the previous section of this thesis this had to be abandoned and instead the model studied was that of N. brasiliensis in the rat.

An initial problem with this model is that rats develop a very high degree of immunity to reinfection after a single infection with several hundred larvae. Thus, after challenge the numbers of adult worms which develop is reduced by around 95% (Jarrett, Jarrett and Urquhart, 1968). Since this degree of immunity is too high to be able to detect immunostimulant properties of an anthelmintic it was first necessary to attempt to establish the number of larvae required in a primary infection that would give only 60% resistance to reinfection in terms of adult worm numbers.

SECTION 2

GENERAL METHODS AND MATERIALS

Experimental animals

All the experiments were conducted using female hooded Lister rats of approximately eight weeks of age and weighing between 150-200 gm (OLAC, Shaws Farm, Bicester, Oxfordshire, England).

All rats were housed in plastic cages with wire mesh tops and floors suspended above sawdust-containing trays. No more than ten rats were housed per cage and the cages were stacked in metal racks. The wire mesh floors prevented rats coming in contact with infected faeces and the cages were washed at regular intervals to prevent any possibility of reinfection of the animals from faecal contamination.

Rats were fed on pelleted diet (Diet 41, John Stewart, Larbert Mill, Larbert, Scotland), this and water being available *ad libitum*. The animal house temperature was maintained at approximately 22°C.

Parasitological techniques

Nippostrongylus brasiliensis infective larvae

The strain of *N. brasiliensis* used in the experiments described here has been maintained in this laboratory for many years by repeated subinoculation in hooded Lister rats.

Culture and harvesting of infective larvae

The technique used for the culture of *N. brasiliensis* infective larvae was as described by Jennings, Mulligan and Urquhart (1963). Faeces were collected from rats with a 7-10 day old infection by placing paper beneath the cages. The faecal pellets were then placed in a mortar with a little warm water and allowed to soak for 15-30 minutes before being broken up and mixed to a paste. Using a spatula a portion of the faecal paste was spread on to the centre of Whatman's No.1 filter papers, diameter 7 cm, the outer edges of the filter papers being kept clear of faeces. The

papers were then moistened by immersing in water and placed on water-saturated foam rubber pads in disposable plastic petri dishes (Sterilin products, Sterilin Ltd., Middlesex, England). After replacing the lids the petri dishes were stacked in a humid incubator at 27°C.

After 4-5 days larvae had usually migrated and could be seen collecting in a fringe around the edges of the filter paper. Larvae were always harvested between 5-10 days after setting up the culture and were used to infect rats on the same day.

Harvesting was accomplished by flooding the petri dishes with water at 37°C, thus allowing the larvae to swim away from the filter paper into the water. After a few minutes the filter paper and foam rubber pads were lifted out and discarded. The water containing the larvae was then collected and filtered through strong filter paper (Whatman 113, 18.5 cm diameter, Whatman Ltd., England) using a Buchner funnel and suction pump. This paper was then placed inverted on an Endecott sieve (mesh No.400, Endecotts Ltd., England) in a Baermann apparatus filled with water at 37°C. The motile larvae migrated into the warm water leaving the faecal debris trapped on the sieve; larvae were subsequently collected from the neck of the funnel.

Larval counting technique and infection of rats

When the required larval dose was more than 100 infective larvae per rat the larval counts were made by a dilution technique as follows. A 1 ml sample of the larval suspension was diluted with water to 10 or 100 ml depending on larval concentration. Forty well mixed 0.025 ml aliquot samples were placed on glass slides and the larvae present counted under a dissecting microscope (Wild model M5, Heerbrugg, Switzerland). The total number of larvae counted was then multiplied by the dilution factor to obtain the

number present in the original suspension, which was then diluted in order that the number of larvae required for infection purposes was contained in 0.1-0.5 ml of suspension.

The above counting procedure was repeated in order to check that the dosing error was less than 10%. Final adjustments in dilution were made if necessary and the required doses withdrawn into 1 ml disposable plastic syringes.

Rats were lightly anaesthetised with Trilene (Trichloroethylene, BDH Chemicals Ltd., Poole, England) and were infected by subcutaneous inoculation of the larvae in the inguinal region using a hypodermic needle (20 G).

When larval doses of less than 100 infective larvae per rat were required the apparatus shown in Figure 1 was used. This consisted of the following parts:

a) A micrometer syringe burette (Type SBU 1a, Radiometer, Copenhagen) with a 5 ml disposable plastic syringe containing a dilute suspension of the infective larvae.

b) A magnetic stirrer placed underneath the syringe containing the larval suspension: this was used at intervals to prevent larvae clumping and sedimenting in the syringe.

c) A 40 cm length of transparent vinyl tubing, size 0.059" x 0.083" - bore x ext. diameter (Portex tubing, Portex Ltd., Hythe, Kent, England) with one end connected to the syringe by a stainless steel hypodermic needle (16 G) and the other end held flattened between two clean glass slides with 3 mm of the tubing projecting. The slides were held firmly together by transparent tape and fixed to the stage of a Projectina microscope (Projectina Co. Ltd., Heerbrugg, Switzerland).

d) An intravenous cannula (Red luer, 5 FG O/D 1.65 mm, length 30 cm, Portex Ltd., Hythe, Kent, England) connected to a

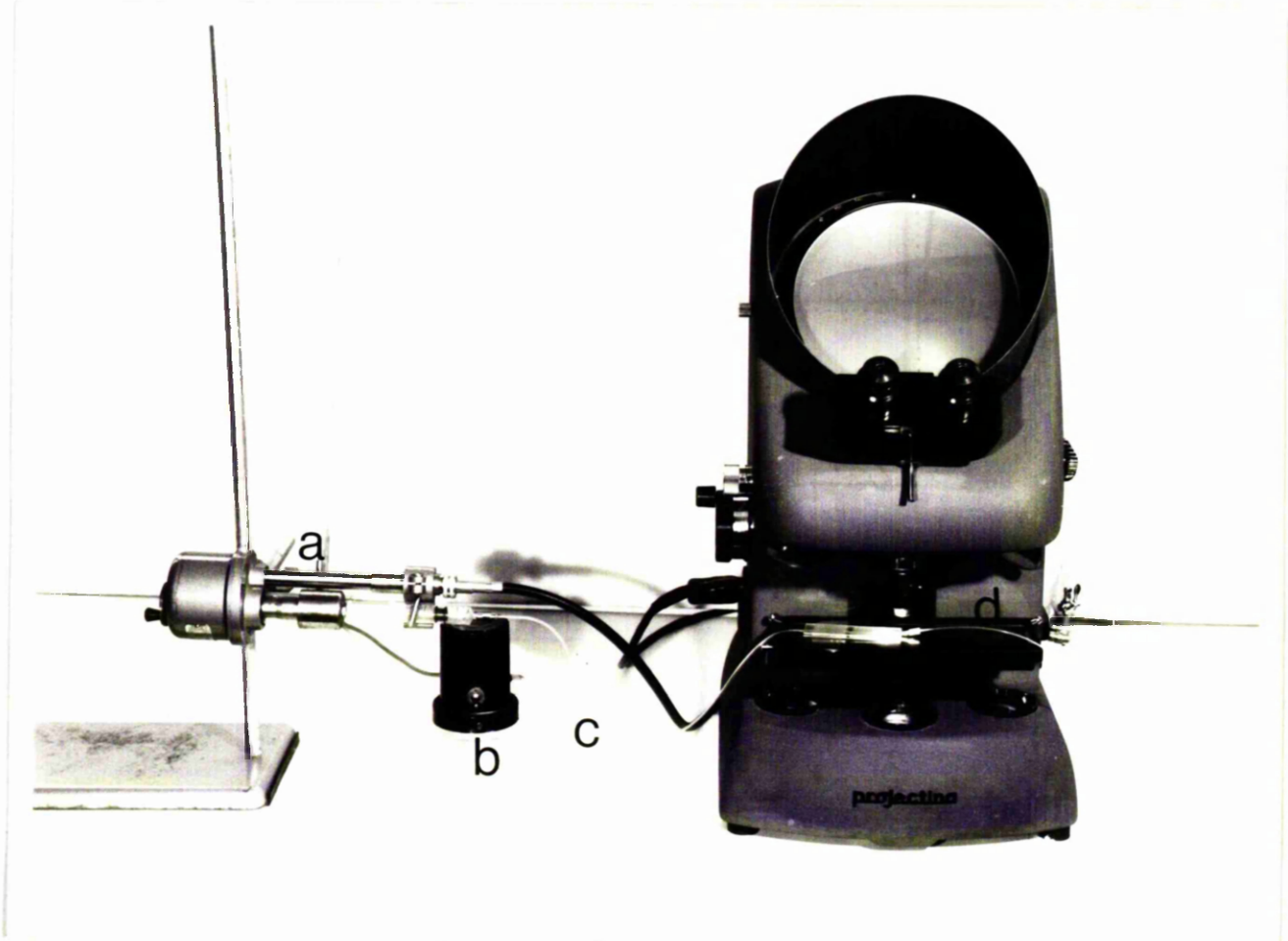

Figure 1

stainless steel one way tap fitted to the side of the microscope as shown in Figure 1. The other end of the cannula was free and could be inserted into the projecting 3 mm of vinyl tubing when required.

The whole arrangement was set up in such a way that the junction of the cannula and vinyl tubing when connected, was directly under the 20 x objective and the image was clearly projected on the screen of the microscope. This greatly facilitated counting of small doses of infective larvae as each larva passing through from syringe to cannula could be observed on the screen.

While counting, the one way tap was kept open and the number of larvae passing through the tubing from the suspension was controlled by gently moving the micrometer handle. When the necessary number of larvae had passed through into the cannula, the tap was closed and the cannula gently removed from the vinyl tubing with the larval suspension held firmly inside by the positive pressure at the open end.

Rats were lightly anaesthetised as mentioned earlier and the larvae inoculated in the inguinal region using a hypodermic needle (20 G) attached to the free end of the cannula. The larval dose was flushed out of the cannula with 0.5 ml normal saline from a 2 ml plastic syringe attached to the one way tap. Using this apparatus there was no difficulty in preparing and administering small doses of larvae accurately and rapidly.

Faecal egg counts

Daily faecal samples were collected from each group of rats by placing paper beneath the cages and these were examined using a modified McMaster technique (Gordon and Whitlock, 1939). In this technique 3 gm of faeces are homogenised in 42 ml of tap water. The resulting mixture is passed through a sieve (Endecotts

Ltd., London, England) of aperture size 250 microns, and 15 ml of the well mixed filtrate transferred to a flat bottomed test tube. After being centrifuged at 2,000 r.p.m. for two minutes the supernatent is discarded and the sediment agitated using a whirlimixer (Fisons Scientific Apparatus, Loughborough, England). The tube is then filled with saturated salt (NaCl) solution and inverted six times to ensure thorough mixing. Sufficient fluid is removed in a pasteur pipette to fill both chambers of a McMaster counting slide (Hawksley & Sons, London, England): as the eggs rise rapidly pipetting must be accomplished quickly.

Under a microscope the number of eggs in both chambers of the McMaster slide are counted and multiplied by 50 to give the total number of eggs per gram (epg) of faeces.

Recovery of worms from the intestine of infected rats

Rats were killed by an overdose of Trilene anaesthesia followed by cervical dislocation. The skin and abdominal wall were incised along the mid ventral line and the entire small intestine immediately removed and slit open longitudinally with blunt scissors. The opened intestine was then placed in a gauze bag suspended in a 250 ml glass beaker filled with luke warm saline. This was then placed in a water bath at 37°C for three hours during which time the worms migrated through the gauze and collected at the bottom of the beaker. As a preservative 5 ml of 10% Formaldehyde Solution (BDH Chemicals Ltd., Poole, England) was added to each beaker.

Worm counting technique

The fluid was carefully decanted from each beaker and the worms counted in petri dishes under a dissecting microscope. Generally all the worms in each sample were counted but when large numbers were present the samples were made up to 50 ml with water, ten 2.5 ml aliquots counted and the total number of worms for each rat then calculated.

Anthelmintics

Thiabendazole (TBZ) - Thiabendazole B Vet C 13.3% w/v suspension (Thibenzole Merck Sharp and Dohme Ltd., Hertfordshire, England) administered orally at a dosage of 100 mg/kg body weight. Each rat received 0.1 ml of suspension.

Levamisole (LEV) - Levamisole hydrochloride BP (Vet) 1.5 w/v solution (Nilverm, Imperial Chemical Industries Ltd., Cheshire, England) administered orally at a dosage of 7.5 mg/kg bodyweight. Each rat received 0.1 ml of the solution.

Fenbendazole (FBZ) - Fenbendazole 10% suspension (Panacur, Hoechst Pharmaceuticals, Middlesex, England) administered orally at a dosage of 7.5 mg/kg body weight. In the case of fenbendazole 1 ml of the original suspension was diluted to 10 ml with distilled water and each rat received 0.15 ml of the diluted suspension.

Statistics

The worm burdens were analysed non-parametrically using the Mann-Whitney U test and probabilities of less than 0.05 were considered significant.

CHAPTER I

THE EFFECT OF ANTHELMINTICS ON THE IMMUNE RESPONSE TO *NIPPOSTRONGYLUS BRASILIENSIS* IN RATS.

Experiment 1 First experimental attempt to produce a 'moderate' degree of immunity to N. brasiliensis with low sensitising infections

The design and results are shown in Table 1. Sixty rats were divided into four equal groups. Groups A, B and C each received a primary infection of 50, 100 and 500 N. brasiliensis L_3 respectively while the rats in Group D remained uninfected. Twenty-four days after the primary infection the rats of all four groups each received 3000 L_3. Five rats from each group were killed on days seven, 10 and 14 after challenge and their intestinal worms recovered. Throughout the experiment group faecal egg counts were monitored.

Results

Worm burdens

Individual worm burdens are given in Appendix B.

On day seven after challenge infection the mean worm burdens of the rats in Groups A, B and C were reduced by 88% - 97% compared with that of the Group D controls and by day 10 the worm burdens were further reduced.

Faecal egg counts

Details are given in Appendix B.

The pattern of faecal egg counts after primary infection of Groups A, B and C reflected the dose of larvae administered with means of around 2000, 4000 and 9000 epg on day eight. Three days later these had dropped to around 50 epg (range 0-50 epg) and remained at a low level, despite challenge, until necropsy. Control Group D rose to a peak of 161,000 epg 11 days after the challenge infection.

Table 1

Groups	No. of rats/ group	Primary infection 'Day 0	Challenge infection Day 24	Mean number of worms (± S E) recovered after challenge		
				Day 7	Day 10	Day 14
A	15	50 L_3	3000 L_3	50 ± 20	2 ± 0.5	16 ± 12
B	15	100 L_3	3000 L_3	191 ± 137	5 ± 3	2 ± 1
C	15	500 L_3	3000 L_3	100 ± 31	1 ± 0.4	10 ± 4
D	15	-	3000 L_3	1546 ± 339	1884 ± 114	268 ± 29

Conclusions

Since the results of this experiment indicated that by day seven post-challenge the protection conferred by a primary infection of 50 L_3 was equal to that induced by 500 L_3 i.e. more than 90% protection, it was considered necessary to reduce the sensitising infection still further to achieve the objective of a 'moderate' immunity.

Experiment 2 Second experimental attempt to produce a 'moderate' degree of immunity to N. brasiliensis with low sensitising infections

In this second experiment the number of larvae used in the sensitising infections was further reduced to between 10 L_3 and 100 L_3 and the post-challenge worm burdens estimated earlier i.e. on days four, seven and 10 after challenge.

Ninety-six rats were used and the design and results are shown in Table 2. Groups E, F, G and H each received a primary infection of 100 L_3, 50 L_3, 25 L_3 and 10 L_3 respectively. Group I rats remained uninfected. On days 11 and 24 two rats from each of Groups E, F, G and H were killed and their intestinal worm burdens estimated to assess the establishment and persistence of parasites from the primary infection. Twenty-four days after primary infection the rats in all five groups each received 3000 L_3. Intestinal worms were recovered and counted from five rats from each group, on days four and seven and from six rats on day 10 after challenge. Throughout the experiment the faecal egg counts were monitored.

Results

Worm burdens

Individual worm burdens are given in Appendix B.

In the rats of Groups E, F, G and H killed on day 11 after primary infection there was a 50%-60% worm establishment and in those killed (at the time of challenge) on day 24, 24%-40% of the initial infection still remained in the intestine.

On day four after challenge, Groups E, F and G showed substantial reductions in worm burdens compared with controls, i.e. between 74%-94%. However, in the Group H rats, which had received a primary infection of 10 larvae prior to challenge, the number of worms

Table 2

Groups	No. of rats/ group	Primary infection Day 0	Challenge infection Day 24	Mean number of worms (± S E) recovered after challenge		
				Day 4	Day 7	Day 10
E	20	100 L_3	3000 L_3	177 ± 82	63 ± 45	23 ± 6
F	20	50 L_3	3000 L_3	496 ± 156	55 ± 13	17 ± 6
G	20	25 L_3	3000 L_3	457 ± 150	144 ± 20	27 ± 14
H	20	10 L_3	3000 L_3	1786 ± 7	273 ± 94	14 ± 6
I	16	-	3000 L_3	1942 ± 64	1388 ± 207	1194 ± 37

which established on day four was similar to the controls ($P = 0.05$). On day seven post-challenge Groups E, F and G showed a 90%-96% and Group H an 80% reduction in mean worm burdens compared with controls. By day 10 the mean worm burdens of all four previously infected groups were reduced by over 98%.

Faecal egg counts

Details are given in Appendix B.

The pattern of faecal egg counts after primary and challenge infections were similar to that observed in Experiment 1.

Conclusions

On the basis of the results obtained with sensitising infections of 10 L_3 i.e. the percentage establishment on day four and 10 were 92% and 20% respectively and since it seemed impractical to reduce the larval dose further it was decided to use this system to study the relationship between anthelmintics and immunity.

Experiment 3 The effect of anthelmintic treatment on the response to reinfection one week later

The design and results of Experiment 3 are given in Table 3. One hundred and seven rats were divided into groups as shown. At the beginning of the experiment the rats in four of these Groups J, K a, L a and M a each received a primary infection of 10 L_3. Seventeen days later four rats from Group J were killed and their worm burdens estimated while the rats in Groups K a, b, L a, b and M a, b received a dose of the anthelmintic indicated. One week after anthelmintic treatment a further four rats from Group J together with two rats from K a, L a and M a were sacrificed while surviving rats in all groups each received a dose of 3000 L_3. On days four, seven and 10 post-challenge five rats from Groups J, K a, L a, M a and N and two rats from Groups K b, L b and M b were killed and their intestinal worm burdens estimated.

Results

Worm burdens

Individual worm burdens are given in Appendix B.

On days 17 and 24 after primary infection necropsy of the four rats from Group J showed that a range of 3-7 and 4-6 worms were present at the time of anthelmintic treatment and challenge respectively. The anthelmintic efficiency of the three drugs at the time of challenge was confirmed in that no worms were recovered from the two rats in Group K a and only one worm was recovered from one of the two rats in Groups L a and M a.

On day four after challenge the mean worm burdens of the Group J rats which received the primary infection and no anthelmintic treatment showed a marked reduction compared with the challenge control Group N ($P < 0.02$). In contrast, the mean worm burdens

Table 3

Groups	No. of rats/ group	Primary infection Day 0	Anthelmintic treatment Day 17	Challenge infection Day 24	Mean number of worms (± S E) recovered in each group after challenge		
					Day 4	Day 7	Day 10
J	23	10 L_3	-	3000 L_3	950 ± 153	244 ± 43	70 ± 17
N	15	-	-	3000 L_3	1819 ± 120	1714 ± 107	1245 ± 152
K a	17	10 L_3	LEV	3000 L_3	1327 ± 179	152 ± 79	11 ± 7
L a	17	10 L_3	TBZ	3000 L_3	1504 ± 102	468 ± 146	20 ± 13
M a	17	10 L_3	FBZ	3000 L_3	1732 ± 110	308 ± 81	12 ± 3
K b	6	-	LEV	3000 L_3	1195	1367	676
L b	6	-	TBZ	3000 L_3	2060	1578	1237
M b	6	-	FBZ	3000 L_3	1858	1875	1558

of the two infected groups (L a and M a) treated one week prior to challenge with TBZ and FBZ respectively were similar to that of the challenge control group. This was not the case with the third infected group treated with levamisole whose worm burden was just significantly lower ($P < 0.05$) and not significantly different from that of Group J.

However, by day seven after challenge the worm burdens of all four groups were substantially reduced compared with the challenge control group i.e. 86%, 91%, 73% and 82% reductions for Groups J, K a, L a and M a respectively. By day 10 the reduction in all four groups was more than 99%.

The worm burdens of the three groups of rats which received only the anthelmintic treatment (K b, L b, M b) one week prior to challenge did not differ markedly from those of the challenge control group with the possible exception of Group K b which received levamisole. For example, on day 10 the worm burden was substantially lower i.e. a mean of 676 compared to 1245 in the controls.

Conclusion

In summary, the results of this experiment indicate that when measured four days post-challenge the immune response of rats was reduced by prior removal of their existing worm burdens with either thiabendazole or fenbendazole. In contrast, treatment with levamisole appeared not to influence the immune response.

However, when measured on days seven and 10 post-challenge the immune response of all three groups was similar to that of the control group which received a primary infection and no anthelmintic treatment before challenge i.e. the efficacy of the immune response was completely restored.

When helminth-naive rats were given an anthelmintic seven days before infection the smallest adult worm burdens were consistently

encountered in the rats given levamisole. However, the numbers of rats in each group were too small to permit statistical evaluation.

It was decided to repeat some of this experiment using a longer time-interval between anthelmintic treatment and challenge. It was hoped that the immune response might not be so marked.

Experiment 4 The effect of anthelmintic treatment on the response to reinfection three weeks later

Experiment 4 is similar in design to Experiment 3 with two exceptions: first, only two anthelmintics were used and secondly, there was an interval of three weeks instead of one week between anthelmintic treatment and challenge.

The design and results of this experiment are given in Table 4. Eighty-one rats were divided into five groups as shown. At the beginning of the experiment the rats in Groups O, P, Q and S received a primary infection of 10 L_3. Seventeen days later rats in Groups P and Q each received a dose of an anthelmintic as shown. The rats in Group S only received the primary infection and on days 17 and 38 after the primary infection and on days four, seven and 10 after challenge, necropsy of three rats from this group on each occasion showed that in the absence of treatment or challenge approximately 30% of the primary infection remained until the end of the experimental period. Five or six rats from each of Groups O, P, Q and R were necropsied on days four, seven and 10 after challenge and their intestinal worm burdens estimated. Throughout the experiment faecal egg counts were monitored.

Results

Worm burdens

Individual worm burdens are given in Appendix B.

The results of the worm burdens were similar to those obtained during the post-challenge period in Experiment 3 in that on day four after challenge there was a significant reduction in the mean group worm burdens of Groups O and P i.e. the control and levamisole groups, compared with the challenge control Group R while the worm burden of the Group Q rats which received

Table 4

Groups	No. of rats/ group	Primary infection Day 0	Anthelmintic treatment Day 17	Challenge infection Day 38	Mean number of worms (± S E) recovered in each group after challenge		
					Day 4	Day 7	Day 10
O	16	10 L_3	-	3000 L_3	1438 ± 259	154 ± 59	1 ± 0.8
P	17	10 L_3	LEV	3000 L_3	1803 ± 247	114 ± 55	4 ± 4
Q	17	10 L_3	TBZ	3000 L_3	2330 ± 67	98 ± 52	11 ± 10
R	15	-	-	3000 L_3	2360 ± 76	1811 ± 99	1322 ± 136
			Mean worm burdens				
S	15	10 L_3	4	5	3	3	4

thiabendazole treatment three weeks before challenge was similar to that of the challenge controls. However, on days seven and 10 all three sensitised groups showed a reduction in worm burdens of between 90-99% compared with controls.

Faecal egg counts

Details are given in Appendix B.

Until the time of anthelmintic treatment the trends in faecal egg output were similar in all four infected groups. While faecal examinations were negative for the treated groups after day 18 and remained so until challenge, the untreated Groups O and S maintained a low egg output over this period. After challenge, the control Group R showed a marked increase in faecal egg counts which rose to a peak of 76650 epg seven days after challenge. Groups P and Q showed a slight rise in egg counts with peak means of 3000 and 1650 epg at day six while low faecal egg counts of the order of 150 epg were recorded from Groups O and S throughout the post-challenge period.

Conclusions

It seemed possible from this experiment that the rapid immune response of the levamisole-treated rats observed on day four post-challenge compared to the thiabendazole-treated rats might be immunomodulatory in origin. The next experiment was concerned with a study of this aspect.

Experiment 5 First experiment to study the immunomodulatory role of anthelmintics

In Experiment 3 the worm burdens of the groups of rats which received anthelmintic treatment with three different anthelmintics without prior infection and a week later the challenge infection did not differ markedly from those of the controls, except for the levamisole-treated group rats which had consistently lower worm burdens than the controls. Because only few rats were involved no definite conclusions could be drawn from these findings. The following experiment was therefore carried out to find whether treatment with levamisole a week before challenge would have any significant effect on worm establishment after infection.

The design and results of this experiment are given in Table 5. Thirty-two rats were divided into two groups of 16. At the beginning of the experiment rats in Group T each received a dose of levamisole and a week later rats in Groups T and U, each received 3000 L_3. Either five or six rats from each group were necropsied on days four, 10 and 14 post-infection and intestinal worm burdens estimated.

Throughout the experiment faecal egg counts were monitored at regular intervals.

Results

Worm burdens

Individual worm burdens are given in Appendix B.

On day four after infection, the mean worm burdens of the rats from both groups were similar while on day 10 the mean worm burdens of Group T rats was lower than that of Group U controls and by day 14 the mean worm burden of Group T rats was significantly lower than that of the Group U controls.

Table 5

Groups	No. of rats/ group	Anthelmintic treatment Day -7	Challenge infection Day 0	Mean number of worms (± S E) recovered after infection		
				Day 4	Day 10	Day 14
T	16	LEV	3000 L_3	557 ± 114	560 ± 75	44 ± 8
U	16	-	3000 L_3	542 ± 57	724 ± 98	181 ± 60

Faecal egg counts

Details are given in Appendix B.

Groups T and U rats gave positive egg counts from day six after infection and showed similar trends till the end of the experiment except that the Group T rats had lower faecal egg counts than the controls from day 11 onwards. On days 11 and 12 the faecal egg counts of Group T were 1250 and 50 respectively while the egg counts of Group U were 13800 and 6200 respectively.

Conclusions

The results of this experiment suggested that there was an early expulsion of the established worms in the levamisole-treated group compared with the controls. However, no definite conclusions could be drawn since the establishment of infective larvae was very low in both groups as judged by the day four worm burdens. Because of this it was decided to repeat the experiment.

Experiment 6 Second experiment to study the immunomodulatory effects of anthelmintics

Experiment 6 is similar in design to Experiment 5 with one exception: there was an additional group which received thiabendazole treatment a week before the infection.

The design and results of this experiment are given in Table 6. Fifty-one rats were divided into three groups of 17. At the beginning of the experiment rats in Groups V and W each received a dose of levamisole and thiabendazole respectively. One week after the anthelmintic treatment rats in Groups V, W and X each received 3000 L_3. Either five or six rats from each group were necropsied on days four, 10 and 14 post-infection and their intestinal worm burdens estimated.

Throughout the experiment faecal egg counts were monitored from all three groups daily.

Results

Worm burdens

Individual worm burdens are given in Appendix B.

There was no significant difference between the worm burdens of all three groups on days four, 10 and 14 after infection.

Faecal egg counts

Details are given in Appendix B.

All three groups gave positive egg counts from day six after infection and showed similar trends till day 10 and from day 11 till the end of the experiment all three groups were negative for worm eggs.

Table 6

Groups	No. of rats/ group	Anthelmintic treatment Day -7	Infection Day 0	Mean number of worms (± S E) recovered at necropsy after infection Day 4	Day 10	Day 14
V	17	LEV	3000 L_3	1081 ± 116	859 ± 76	31 ± 15
W	17	TBZ	3000 L_3	1034 ± 91	952 ± 65	36 ± 11
Z	17	-	3000 L_3	1087 ± 99	954 ± 36	30 ± 12

Conclusions

The results of this experiment indicated that anthelmintic treatment with either levamisole or thiabendazole one week before infection did not in any way alter the immune expulsion of established worms. This finding is in contrast to the results obtained in Experiment 5, where there was an indication of early expulsion of the worm burdens in levamisole-treated rats. This discrepancy may be due to the fact that the establishment of infective larvae was low in Experiment 5 and high in Experiment 6 and when establishment is high any immunomodulatory anthelmintic effect is masked by a more efficient immune response of the host.

DISCUSSION

These experiments were undertaken to find if the elimination of an adult infection of N. brasiliensis in rats by anthelmintic treatment was associated with any alteration in the degree of immunity to subsequent infection. In the event that this was so it was hoped to establish whether this was due to the removal of the residual worm burden of the sensitising infection or to an immunomodulatory effect of the anthelmintic.

Because even a single N. brasiliensis infection is invariably followed by a very high degree of immunity it was first necessary to attempt to modify the sensitising infection to produce a more modest degree of immunity.

The results of the first experiment indicated that this might be difficult since it was evident that by day seven post-challenge the protection conferred by a primary infection of 50 L_3 was equal to that induced by 500 L_3 both resulting in more than 90% reduction in establishment of the challenge infection.

In the second experiment, doses of 10-100 L_3 were used in the sensitising infections and the post-challenge worm burdens estimated from day four onwards in order to detect any loss of immunity in the early stages of the challenge infection. The three groups of rats which received a primary infection of 25, 50 and 100 L_3 respectively had significantly lower worm burdens than the challenge controls on day four post-challenge. However, the numbers of worms in the group which received a sensitising infection of 10 L_3 was similar to that of the challenge control group. Nevertheless, by day seven post-challenge, the mean worm burdens of all four immunised groups were significantly lower than those of the controls and indeed by day 10 they were indistinguishable from each other.

This indicated the striking fact that although the immunity conferred by a single infection of only 10 L_3 did not prevent the initial establishment of worms from a challenge infection of 3000 L_3 it resulted in their rapid expulsion from the small intestine so that six days later the worm burdens were negligible. The immunogenicity of such a small number of larvae is remarkable and perhaps without parallel in other helminth species.

It was then decided to explore the possible effect of anthelmintic treatment on the immunity conferred by this very light infection.

In the first of these experiments anthelmintic treatment was given on day 17 after infection i.e. seven days before challenge. Two of the three anthelmintics used, thiabendazole and fenbendazole, had a similar effect i.e. four days after challenge the numbers of worms in the treated groups were not significantly different from those of the challenge control group. The group which received the third anthelmintic - LEV had the fewest worms and the mean worm burden of this group was not significantly different from the sensitised, untreated and challenged group. However, the full efficiency of the immune response in all the drug-treated groups was quickly expressed and by days seven and 10 after challenge the degree of expulsion was similar to that of the untreated group which had received the sensitising infection.

In the second experiment, the interval between treatment and challenge was increased to two weeks. The results were similar to those of the previous experiment i.e. with the exception of the levamisole-treated group, a delay in the expression of immunity until seven days after challenge. Thereafter the expression of immunity was complete and similar to that of the sensitised and untreated group of rats.

In conclusion, it seems that the removal of light residual

worm burdens of N. brasiliensis in rats with either thiabendazole or fenbendazole does not affect the full expression of the immune response by the seventh to the tenth day following challenge. However, it does delay this expression for at least four days following the challenge.

When levamisole is used this delay is less in evidence and the results, bordering on the edge of significance but consistent, suggest that levamisole might act as an immunostimulant.

CHAPTER II

THE EFFECT OF ANTHELMINTICS ON THE IMMUNE RESPONSE TO TRANSPLANTED ADULT *NIPPOSTRONGYLUS BRASILIENSIS* INFECTION IN RATS.

INTRODUCTION

In the previous chapter it was shown that under certain circumstances levamisole had a marginal, although statistically significant, immunostimulant effect in N. brasiliensis infection of the rat. This was apparent when levamisole was administered as an anthelmintic to remove worms from a sensitising infection prior to heavy larval challenge. In this instance there was a reduction in worm establishment which was not observed with other anthelmintics. However, when levamisole was simply administered prior to a primary infection this effect was inconsistent in that in one of three experiments their was no effect on subsequent worm establishment.

One problem in extrapolating this result to gastro-intestinal nematode infections of ruminants is the fact that the life-cycle of N. brasiliensis has a migratory phase involving the vascular and pulmonary systems. In contrast, the parasitic life-cycles of these nematodes of ruminants takes place entirely on or in the gastro-intestinal mucosa. It could be argued that if the immuno-stimulant properties of levamisole affects only the migratory phase of N. brasiliensis then the result is perhaps irrelevant in ruminant infections.

It was considered that one way in which this aspect might be studied was to transplant adult N. brasiliensis worms into the duodenum of rats thus circumventing the somatic migration. There is evidence in the literature that such infections stimulate a good degree of immunity (Ogilvie, 1962, 1965) in that expulsion of the transplanted infection occurs within a week or so the rats being subsequently immune to larval or adult challenge. Ogilvie found that adult female worms were a primary source of protective antigen and that the immunity stimulated was active against both

larval stages and adults. She concluded that in order to achieve a level of protection similar to that conferred by five female worms, 1000 L_3 were needed when the larval infection was terminated by anthelmintic treatment at 96 hours.

This chapter records the results of an experiment in which rats were infected and subsequently challenged with transplanted adult worms and the effect of anthelmintic abrogation of the sensitising infection. It also describes a simple and non-surgical technique for transplanting adult worms which is easy to perform and non-traumatic for the recipient even when repeated infection is necessary.

TRANSFER OF ADULT WORMS BY GASTRIC INTUBATION

In studies where adult N. brasiliensis were transplanted from donor rats to recipient hosts, except for a report by Spindler (1936) all other workers used surgical procedures and directly transplanted worms into the intestine of the recipient.

Spindler (1936) administered adult worms to previously uninfected rats under ether anaesthesia using a duodenal tube. Following administration he recorded positive egg counts from most of the infected rats and from the results obtained after challenging these rats subcutaneously with 500 L_3 he concluded that "..... the presence of adult worms in the intestines of the host animals produced a resistance of a sort that inhibited to some extent the egg production of the challenge worm burdens..... and the inhibition of development of the larvae from the challenge infection in the lungs is associated with a prior passage of larvae through these organs".

Since transfer of adult worms by gastric intubation is convenient and less disturbing to recipients than surgical transfer it was decided to use the following method for the transfer of adult worms.

Method

Donor rats infected five days earlier with 3000-4000 N. brasiliensis infective larvae were killed by an overdose of "Trilene" anaesthesia followed by cervical dislocation. The skin and abdominal wall were incised along the mid ventral line and the entire small intestine slit open longitudinally with blunt scissors. The opened intestine was then placed in a gauze bag suspended in a 250 ml glass beaker filled with lukewarm saline. This was then placed in a water bath at 37°C. The worms were collected after 30 minutes incubation were allowed to sediment and the concentration adjusted so that the number of worms to be transferred to each rat was contained in a volume of between 2 and 5 ml. The suspension was kept constantly mixed in a water bath at 37°C to ensure even distribution of worms. The appropriate volume was withdrawn into a 5 ml disposable plastic syringe with an attached 3 cm length of intravenous catheter (Red luer, 5 F G O/D 1.65 mm, Portex Ltd., Kent, England). The worm suspension was carefully administered to rats orally under light anaesthesia the catheter functioning as a stomach tube.

Transfers of adult worms were made as quickly as possible and care was taken to subject worms to the minimum of mechanical damage.

A pilot study was first carried out in which eight adult parasite-free rats were each given 300 five day old (D_5) N. brasiliensis by gastric intubation, killed three days later and their worm burdens estimated. Positive egg counts were recorded in all the infected rats from the day after worm transfer and the worms recovered at necropsy from each rat indicated that 50% - 60% of the intubated worms had established in the small intestine of the recipients.

Experiment 7 Do anthelmintics have immunomodulatory properties when the sensitising and challenge infections are adult worms transplanted into the duodenum?

The design and results of Experiment 7 are given in Table 7. Forty-one rats were divided into five groups as shown. A further thirty rats were used as donors to produce day five (D5) worms.

At the beginning of the experiment the rats in Groups A, B, C and E each received a primary infection of 20, D5 worms. Group D rats remained uninfected. Twelve days after worm transfer three rats from Group E were sacrificed and their worm burdens counted. At the same time the rats in Groups B and C each received a dose of levamisole and thiabendazole respectively. One week after anthelmintic treatment a further three rats from Group E were necropsied and the rats in Groups A, B, C and D each received 600 D5 worms.

Five days after challenge all five groups of rats were killed and their intestinal worms recovered. Throughout the experiment faecal egg counts were monitored at regular intervals.

Results

Worm burdens

Individual worm burdens are presented in Appendix B.

On days 12 and 19 after primary infection necropsy of the three rats from Group E showed that a range of 7-17 and 4-11 worms were present at the time of anthelmintic treatment and challenge respectively. From the seven rats killed from this group on day five post-challenge a mean of eight worms were recovered (range 2-14). At the end of the experiment the worm burdens of all three preimmunised groups were similar and showed a 50-58% reduction compared with the controls.

Table 7

Groups	No. of rats/ group	Primary infection Day 0	Anthelmintic treatment Day 12	Challenge infection Day 19	Mean worm burdens (± S E) at necropsy after challenge infection Day 5	Statistical significance
A	7	20 worms	-	600 worms	106 ± 16	A vs B NS A vs C NS A vs D S (P = .003)
B	7	20 worms	LEV	600 worms	88 ± 18	B vs C NS B vs D S (P = .001)
C	7	20 worms	TBZ	600 worms	89 ± 20	C vs D S (P = .002)
D	7	-	-	600 worms	212 ± 24	
				Mean worm burdens		
E	13	20 worms	11	7	8	

NS Not significant
S Significant

Faecal egg counts

Details are given in Appendix B.

Until day 13 after the primary worm transfers the pattern of faecal egg counts of Groups A, B, C and E were similar with mean peak counts of around 2000, 900, 900 and 1000 epg on day two. Faecal examinations were negative for the treated groups after day 14 and remained so until challenge. The untreated Groups A and E maintained a low egg output over this period. After challenge the control group showed a marked increase in faecal egg output which rose to a peak of 9800 on day four after challenge while Groups A, B and C only showed a rise in the egg counts on day one post-challenge with means of 2000, 1250 and 1050 epg. Low faecal egg counts were recorded from Group E rats throughout the post-challenge period.

Conclusions

These results show that sensitising infections of 20 transferred adult worms are able to confer a significant degree of immunity to an adult worm challenge administered 19 days later since, in the sensitised rats 50 to 58% of the challenge worms had been expelled five days later compared with the controls.

Also clear is the fact that the use of either of two anthelmintics, levamisole and thiabendazole, 12 days after the primary infection did not significantly affect the immune response one way or the other providing further evidence of the absence of any drug-induced immunomodulatory effect.

However, the degree of immunity observed is poor compared with that stimulated by subcutaneous infection with 10 L_3 (See Section 2, Chapter I). Two possible explanations for this are, first, that the rats given the transferred adult infection were in fact highly immunised in consequence but that the challenge

infection of adult worms had by-passed or evaded a significant part of the immune mechanism. Alternatively, it is possible that 20 adult worms are simply poorly immunogenic. These two questions are examined in the next chapter.

Discussion

This experiment was undertaken to find out whether the immunomodulatory effects of levamisole observed in the previous chapter (Chapter 1), where there was a reduction in the establishment of subsequent larval infection when a sensitising larval infection was eliminated by this drug will be evident when the sensitising and the challenge infections consisted of non-migratory stages transferred directly into the intestines.

Our study indicates that gastric intubation is a successful method to transfer adult worms and that 20 adult worms transferred in this manner were able to confer significant protection against subsequent challenge with 600 adult worms. But this protection was less marked than that produced by small numbers of larvae (10 L_3) against a high larval challenge.

Removal of the sensitising adult worms by either of the two anthelmintics, levamisole or thiabendazole a week prior to challenge did not alter the degree of protection since the worm burdens of sensitised and untreated controls were similar to those of the treated groups by day five following challenge. The apparent immunostimulant effect of levamisole evident in the previous experiments (Chapter 1) using larval challenge was not observed in this experiment. The reasons for this are not clear but may be due to a number of factors including the developmental stage of the challenge infections. In previous reports where immunostimulant properties were attributed to levamisole when used as an anthelmintic the parasites involved had migratory or tissue phases (Oakley, 1981; Mitchell and Armour, 1981) or the hosts were malnourished and thus could be immunocompromised (Duncombe et al, 1979).

It seems probable from the results obtained from this study that the immunomodulatory properties of levamisole are not evident in non-migratory parasite infections.

CHAPTER III

THE ROLE OF LARVAE AND ADULT WORMS IN THE IMMUNE RESPONSE TO *NIPPOSTRONGYLUS BRASILIENSIS*.

INTRODUCTION

In the previous chapter it was shown that a transferred infection of 20 adult worms conferred some degree of immunity to a challenge of 600 adult worms which approached a 60% reduction in worm burden. In contrast, it was previously demonstrated (Chapter I) that as few as 10 larvae stimulated a very high degree of immunity, of the order of 95%, to a challenge infection of 3000 larvae. In view of the potential antigenic mass of the adult worms this result was perhaps surprising.

There are at least three possible reasons for this degree of disparity in protection.

First, the adult worms, in spite of their bulk, are, in fact, relatively poor sources of antigen.

Second, the lumen of the intestine may be a relatively poor site for the uptake of protective antigens and their diffusion to the immunological apparatus; in consequence the immune response is poor.

Thirdly, when a challenge infection is transplanted directly into the intestine the immune response is largely evaded.

The answers to these questions depend on knowledge of the origin and nature of the protective antigens and an appreciation of the mechanisms involved in the immune response to N. brasiliensis. Unfortunately, clear answers to both of these questions are not known despite a vast amount of published work. Although a comprehensive review of the relevant literature is beyond the scope of this thesis there follows a brief review which deals with some of the most important features of the immune response to N. brasiliensis.

Parasite antigens

The nature of the protective antigens is still unknown.

In fact, the problem of isolating these is extremely complex since antigenic material may be associated with feeding, excretion, cuticular surfaces etc. and the spectrum of these may change as the parasite grows and moults. To date, some of the antigens which have been studied are:-

i) Acetylcholinesterase; this is present in the secretions of nematodes and has been proposed as a protective antigen on the basis that the enzyme may act as a biochemical holdfast for adult worms in the intestine by inhibiiting local neuromuscular activity. Jones and Ogilvie (1972) provided some evidence which suggested that host antibodies might modulate enzyme production in favour of the host. However, working with Trichostrongylus colubriformis in the guinea-pig Rothwell and Merritt (1975) were able to induce protection using antigen fractions without acetylcholinesterase activity.

ii) Nematode allergens have been extensively studied as protective antigens in view of their remarkable ability to stimulate IgE antibodies but as yet the role of these antibodies in the immune response is still open to debate (Jones and Ogilvie, 1967; Murray, 1972).

iii) Trichuroid worms possess a glandular structure around the oesophagus called a stichosome and Jenkins and Wakelin (1977) have identified a protein secreted by this organ which induced a high degree of immunity in mice. However, this structure is not present in N. brasiliensis.

iv) Recently, using surface labelling of the cuticle of N. brasiliensis with ^{125}I, followed by detergent solubilization and then electrophoretic and radioimmunoprecipitation procedures, Maizels and Ogilvie (1980) have demonstrated stage-specific cuticular antigens whose spectrum is relatively simple being composed of a

polymer matrix of only two sub-units (Ogilvie and Philip, 1980). These antigens are thought to be responsible for adherence and cytotoxicity of eosinophils which has been demonstrated in in vitro studies (MacKenzie, Preston and Ogilvie, 1978). However, the role of these antigens in the immune response in vivo is still unknown.

Immune Mechanisms

The vast literature on this subject has, so far, failed to provide an explanation of the mechanism of immune expulsion of N. brasiliensis which is acceptable to the majority of workers.

The immune expulsion of a primary infection is entirely dependent on events on and within the intestinal mucosa and from examination of results obtained from studies on the kinetics of worm establishment in first reinfections (Jarrett, Jarrett and Urquhart, 1968) it is likely that a similar situation occurs there also. However, in rats subjected to repeated reinfection it seems likely that a significant number of larvae are destroyed in the lungs and this is supported by studies of Love, Kelly and Dineen (1974) with challenge infections of hyperimmune rats.

It seems not unreasonable to consider, on the basis of the in vitro studies reported by MacKenzie et al (1978), that such larvae might be destroyed by an antibody dependent cell cytotoxicity reaction primarily involving eosinophils.

What occurs in the intestine is less easy to understand. Early studies showing a significant expulsion of adult worms transferred into rats passively immunised with immune serum indicated a relatively straightforward mechanism (Mulligan, Urquhart, Jennings and Neilson, 1965).

It was subsequently suggested that in rats infected with larvae such expulsion would be accelerated by the existence of a state of local anaphylaxis in the intestinal mucosa which by

inducing hyperaemia and vascular permeability would facilitate the transfer of anti-worm antibody to the mucosal surface (Urquhart, Mulligan, Eadie and Jennings, 1965; Barth, Jarrett and Urquhart, 1966).

The anaphylaxis was shown to be associated with hyperplasia of intestinal mast cells and globule leukocytes and a high titre of specific IgE antibody with the subsequent degranulation of the mast cells and liberation of vaso-active amines (Miller, 1971; Murray, 1972; Askenase, 1980).

However, it was subsequently shown that pre-treatment of mice with anti - μ antiserum which severely reduced serum immunoglobins including IgE, did not affect the expulsion of adult N. brasiliensis worms (Jacobson, Reed and Manning, 1977). It was also found that mice, congenitally deficient in mast cells were able to expel their worm burdens in a relatively normal fashion (Uber, Roth and Levy, 1980).

Interest then switched to the fact that thoracic duct lymphocytes (TDL), which had no immunoglobulin on their surface i.e. presumptive evidence of T cells, when taken from infected rats and transferred to irradiated recipients could accelerate the expulsion of a transplanted population of 'antibody-damaged' adult worms (Ogilvie, Love, Jarra and Brown, 1977). How expulsion was effected was not established.

Recently Miller and his co-workers (Miller and Nawa, 1979a; Miller, Nawa and Parish, 1979) have shown that the transfer of Ig negative TDL from immune rats into infected recipients accelerates the production of goblet cell hyperplasia and mucus production in the intestine. More recently Miller and Nawa (1979b) have demonstrated that a factor or factors in immune serum also stimulate this response. These observations have led them to conclude that mucus 'trapping'

and exclusion of the challenge infection from the intestinal surface may play an important role in the immune response and that the role of the sensitised T cell may be to stimulate the differentiation and proliferation of goblet cells. They do not, however, rule out the cooperative action of worm antibody, IgE, mast cells and intestinal anaphylaxis (Miller, Huntley and Wallace, 1981).

Other mechanisms involving such factors as prostaglandins (Dineen, Kelly, Goodrich and Smith, 1974; Richards, Bryant, Kelly, Windon and Dineen, 1977) IgA (Poulain, Luffau and Pery, 1976; Sinski and Holmes, 1977) and macrophages (see review by Befus and Bienenstock, 1981) have also been invoked by different workers as playing a significant role in the immune response.

It seems likely that the total phenomenon of immune expulsion probably involves several mechanisms which may operate cooperatively, sequentially or independently.

The three experiments reported in this chapter are concerned primarily with the relative immunogenicity of larval and adult infections of N. brasiliensis and were prompted by the relatively poor degree of protection observed in the experiment of the previous chapter (Expt. 7). In this, rats immunised with a primary infection of 20 transferred adult worms were only able to stimulate a degree of protection of the order of a 50-60% reduction when challenged 19 days later with 600 transferred adult worms. This contrasts markedly with the 95% protection observed consistently in earlier experiments when rats immunised with 10 larvae were subsequently challenged with 3000 larvae and seems at variance with the conclusion of Ogilvie (1965) that "Immunity to N. brasiliensis in rats is stimulated primarily by the adult worms".

Experiment 8 Does a challenge infection of transferred adult worms evade part of the immune response?

This experiment was designed to answer the first possibility raised by Experiment 7 in the last section, that is, the possibility that a challenge infection of transferred adult worms may evade the immune mechanism to a significant extent.

The design of this experiment is given in Table 8. Forty-five rats were divided into six groups as shown. At the beginning of the experiment rats in Groups A, B, C, D and E each received a primary infection consisting of 10 L_3 and Group F rats remained uninfected.

One day after the primary infection rats in Group E each received a dose of fenbendazole (7.5 mg/kg). On day four, fenbendazole was administered to the rats in Group D. One week after the sensitising infection five rats from Group C were killed and their intestinal worm burdens estimated, while the remaining rats in Group C received anthelmintic treatment. On day 17, five rats in Group B were sacrificed and their intestinal worms counted while the surviving rats in this group each received fenbendazole.

Twenty-four days after the sensitising infection three rats from Group A were sacrificed and their intestinal worm burdens estimated while the surviving rats in all groups each received 1500 adult (D5) worms by gastric intubation; these adult worms were harvested from rats which received infective larvae five days earlier.

On day seven post-challenge the rats from all six groups were sacrificed and their intestinal worm burdens estimated.

Throughout the experiment faecal egg counts were monitored at regular intervals.

Table 8

Groups	No. of rats/ group	Primary infection Day 0	Anthelmintic treatment Post infection D 1	D 4	D 7	D 17	Challenge infection Day 24	No. of rats sacrificed from each group Day 7
A	8	10 L_3	-	-	-	-	1500 worms 3*	5
B	10	10 L_3	-	-	-	FBZ 5*	1500 worms	5
C	10	10 L_3	-	-	FBZ 5*	-	1500 worms	5
D	6	10 L_3	-	FBZ	-	-	1500 worms	5
E	6	10 L_3	FBZ	-	-	-	1500 worms	5
F	5	-	-	-	-	-	1500 worms	5

*No. of rats from group necropsied on that day

Results

Worm burdens

Individual worm burdens are presented in Appendix B and the mean worm burdens are given in Table 9.

On days seven, 17 and 24 after primary infection with 10 L_3 necropsy of five, five and three rats from Groups C, B and A respectively showed that a mean of five worms were present on days seven and 17 and a mean of four worms on the day of challenge.

The worm burdens of all the preimmunised groups showed significant reductions of between 86-96% compared with controls on day seven post-challenge. Group E which received anthelmintic treatment on day one after the sensitising infection had the highest mean worm burden of the preimmunised groups (i.e. 59 worms).

Faecal egg counts

Detailed results are presented in Appendix B.

After primary infection the faecal egg counts of Groups B, C and D which received FBZ on days four, seven and 17 respectively showed that FBZ treatment completely prevented the appearance of eggs after treatment. However in Group E which was given FBZ one day after infection small numbers of eggs (50 - 150 epg) were present between days seven and 10.

After challenge with adult worms small numbers of eggs ranging from 0 - 4000 epg were recorded in the immunised groups compared with 2500 - 23400 epg in the control group.

Conclusions

The results of this experiment show clearly that a challenge infection of transferred adult worms cannot evade the immune mechanism and that as few as 10 L_3 will stimulate a degree of protection of the order of 95%. Even when FBZ was given one day after the

Table 9

Groups	Mean worm burdens (± S E) at necropsy			
	Post-infection			Post-challenge
	D 7	D 17	D 24	D 7
A	-	-	4	21 ± 9
B	-	5	-	21 ± 9
C	5	-	-	34 ± 17
D	-	-	-	18 ± 10
E	-	-	-	59 ± 25
F	-	-	-	425 ± 29

larval infection the degree of protection was 86%; when FBZ was given on a single occasion at four, seven or 17 days after infection the order of protection was similarly high i.e. 96%, 92% and 95% respectively.

Experiment 9 Is a sensitising infection of transferred adult worms a relatively poor immunogenic stimulus compared with that provided by a larval infection?

This experiment was undertaken to study the second possibility raised by Experiment 7 in the last chapter i.e. that a primary infection of adult worms may be an inferior antigenic stimulus compared with a larval infection.

The design and results of Experiment 9 are given in Table 10. Twenty-six rats were divided into five groups as shown. At the beginning of the experiment the rats in Groups G, H and I each received a sensitising infection of 300 D5 worms by gastric intubation. Twenty-four days after the primary infection all four rats in Group G were sacrificed and their worm burdens estimated. At the same time the rats in Groups H and J each received 800 D5 worms while the rats in Groups I and K each received 3000 L_3. Seven days after challenge all the rats were sacrificed and their intestinal worm burdens estimated.

Throughout the experiment faecal egg counts were monitored at regular intervals.

Results

Worm burdens

Individual worm burdens are given in Appendix B.

A mean of six worms were recovered from the four rats necropsied from Group G at the time of challenge. Seven days after challenge with 800 worms the sensitised rats in Group H had significantly lower worm burdens than their Group J controls, i.e. a reduction of 89%. The mean worm burdens of the sensitised Group I rats which received a challenge infection of 3000 L_3 were reduced by 99% when compared with their controls in Group K.

Table 10

Groups	No. of rats/ group	Primary infection Day 0	Challenge infection Day 24	Mean number of worms (± S E) recovered at necropsy after challenge infection Day 7
G	4	300 worms	6 (mean worm burden)	-
H	5	300 worms	800 worms	18 ± 6
I	5	300 worms	3000 L_3	10 ± 5
J	6	-	800 worms	163 ± 9
K	6	-	3000 L_3	1209 ± 67

Faecal egg counts

These, recorded in Appendix B, show that the primary transplanted infection was successful in that four or five days post-infection mean group egg counts ranging between 4000 and 11000 epg were recorded. After challenge egg counts of between 0 - 150 epg were recorded in the pre-infected groups compared with levels of 15000 and 45000 epg four or five days after infection in the control groups.

Conclusions

This experiment, within the limitations of the group sizes of five or six rats, shows that 300 adult worms are capable of stimulating a high degree of immunity, approaching 90%, against a challenge infection of either 3000 larvae or 800 adults. This level of immunity is similar to that conferred by an immunising infection of 10 L_3 (see Chapter I) and one must conclude that adult worms are perfectly capable of stimulating a completely effective immune response. However, on the results of this experiment and the two reported previously they are relatively poorly antigenic since many more adult worms are required to give the same response as 10 larvae.

Experiment 10 The response of immunised rats to low levels of challenge with N. brasiliensis

The previous experiments in this section have shown that rats infected with 10 larvae given subcutaneously or with 300 transferred adult worms develop a marked immunity, of the order of 80-90%, to a challenge infection of either 3000 larvae or 800 transferred adult worms.

The aim of the present experiment was to find out whether a similar protective response would operate in the face of a marked reduction in the challenge infection to 300 L_3 and 30 L_3.

The design and results of Experiment 10 are given in Table 11. Eighty-two rats were divided into eight groups as shown.

At the beginning of the experiment rats in Groups L, O and R each received a primary infection of 10 L_3 while the rats in Groups M, P and S were each given 250, D_5 worms. Rats in Group N and Q remained uninfected during this period. Twenty-four days after primary infection the rats in Groups L, M and N each received a challenge infection consisting of 300 L_3 while the rats in Groups O, P and Q each received 30 L_3; five rats from each of the unchallenged Groups R and S were necropsied at this time and their worm burdens estimated.

On days seven and 10 after challenge the given number of rats from each group were sacrificed and their worm burdens estimated.

Throughout the experiment faecal egg counts were monitored at regular intervals.

Results

Worm burdens

Individual worm burdens are presented in Appendix B.

At the time of challenge a mean of four and nine worms

Table 11

Groups	No. or rats/ group	Primary infection Day 0	Challenge infection Day 24	No. of rats sacrificed Post-challenge Day 7	Day 10	Mean worm burdens (± S E) at necropsy Post-challenge Day 7	Day 10
L	10	10 L_3	300 L_3	5	5	16 ± 3	21 ± 5
M	11	250 worms	300 L_3	6	5	14 ± 4	12 ± 4
N	10	-	300 L_3	5	5	98 ± 6	80 ± 12
O	10	10 L_3	30 L_3	5	5	13 ± 2	15 ± 2
P	11	250 worms	30 L_3	6	5	3 ± 1	4 ± 1
Q	10	-	30 L_3	5	5	16 ± 1	17 ± 1

						Mean worm burdens Post-challenge		
						Day 24	Day 7	Day 10
R	9	10 L_3	-	2	2	4	3	3
S	11	250 worms	-	3	3	9	9	5

were present in the rats necropsied from the unchallenged Groups R and S respectively. In the rats from these groups necropsied after challenge, a mean of three worms were recovered on both day seven and 10 from Group R while a mean of nine and five worms were recovered from the Group S rats on these days respectively.

Rats from Groups L and M which received a challenge of 300 L_3 had significantly lower mean worm burdens on days seven and 10 post-challenge when compared with controls in Group N, i.e. reductions of 84% and 86% respectively on day seven and reductions of 74% and 85% respectively on day 10.

The mean worm burdens of Group O rats which were sensitised with 10 L_3 and subsequently received a challenge of 30 L_3 did not differ significantly from that of the controls in Group Q either on day seven or day 10 post-challenge. In contrast, the Group P rats which had received a primary infection of 250 adult worms prior to challenge with 30 L_3 had significantly lower worm burdens than the controls with reductions of 81% and 76% on days seven and 10 post-challenge respectively.

Faecal egg counts

Details are presented in Appendix B and show that the primary infection established satisfactorily in all infected groups. After challenge in all the immunised groups, egg counts ranging between 50 and 600 epg were recorded six and eight days post-challenge. In the control Groups N and Q the egg counts were around 3000 and 1000 epg respectively on the same days.

Conclusions

These results suggest that even when the level of challenge is reduced to 300 L_3 the immune response is still capable of operating satisfactorily. When the challenge is reduced still further to

30 L_3 the rats immunised by an adult infection still respond adequately but, perhaps rather surprisingly, the rats immunised by larval infection fail to respond properly and allow the bulk of the larval challenge to develop.

Discussion

The three experiments described in this chapter were stimulated by the observation that a transferred adult infection of 20 *N. brasiliensis* only conferred a degree of protection of the order of 50-60% when the rats were challenged with a second infection of 600 adult worms; this contrasted strongly with the 95% protection conferred by a subcutaneous infection of 10 L_3 against a challenge of 3000 L_3. These results rather suggested that either the challenge infection of adult worms, intubated directly into the stomach, had evaded the immune response or, alternatively, that the adult worms were relatively poorly immunogenic. This chapter is concerned primarily with clarification of this question.

Previous work in this particular area is largely confined to that of Ogilvie (1965) who studied the results of a series of experiments in which rats were immunised with adult worms and challenged by subcutaneous injections of L_3. Working with sensitising infections of from 800 adult worms to as few as 10 female worms she obtained a degree of protection of around 90% and therefore concluded that immunity was stimulated primarily by adult worms, particularly females.

A similar observation 'that the intestinal phase is of great immunological importance' was made by Prochazka and Mulligan (1965) who worked with larval infections where intestinal development was truncated by X - irradiation of the larvae.

This conclusion of Ogilvie's i.e. that adult worms are completely effective as immunogens when the rats are subject to larval challenge is confirmed by the results reported here in Expts. 9 and 10 where rats immunised with 250-300 adult worms were around 90% resistant to a challenge infection of 300/3000 larvae.

When, however, such rats are challenged by a transferred infection of adult worms the degree of immunity seems to be dependent on the size of the sensitising worm infection. Thus, after a sensitising infection of 300 adult worms, the degree of protection was 95% against 800 adult worms (Exp. 9); this compares favourably with the previous finding that 10 L_3 gives 95% protection against a transferred infection of 1500 adults. In contrast, after a sensitising infection of only 20 adult worms the degree of protection is only around 50-60% after a challenge of 600 adult worms (Exp.7).

These results show that transferred adult worms can confer a high degree of protection against a subsequent challenge of adult worms i.e. the sensitising infection is immunogenic and the challenge infection cannot evade the immune response. However, they also suggest that sensitising infections of adult worms are not as immunogenic as a comparable number of larvae given subcutaneously.

In this connection, it is interesting that Ogilvie (1965) using 10 female worms as a sensitising infection observed 90% protection against a challenge infection of 500 larvae. It seems that the degree of immunity is boosted if either the sensitising infection or the challenge infection is composed of larvae which are allowed to migrate. Perhaps the direct access of antigen to the tissues during this phase has an 'immunostimulant' effect either on primary or secondary responses. Apparently this effect is even evident when a larval infection is truncated by an anthelmintic as soon as one day after infection.

In the final experiment described here an attempt was made to find the protection conferred by 10 L_3 or 250 transferred adult worms to low challenge infections consisting of either 300 L_3 or 30 L_3. It was evident from the results that rats sensitised with 10 L_3 or 250 adults were resistant to challenge with 300 L_3

in that they harboured significantly lower worm burdens than the appropriate challenge controls on days seven and 10 post-challenge. However, when the challenge infection was further reduced to 30 L_3 the two sensitised groups responded differently. Rats which had received 250 worms showed protection, in terms of challenge worm burdens, while the groups sensitised with 10 L_3 harboured worm burdens similar to those of the challenge controls both on days seven and day 10 after challenge.

There are few reports on the immunising effect of small infections. Jenkins and Phillipson (1970) observed that rats subjected to trickle infection, consisting of five larvae daily over a period of weeks, did not elicit an immune response with expulsion typical of that which follows a single large larval infection. Instead the worms remained in the small intestine of the host for a period of several months. Later the same authors (Jenkins and Phillipson, 1972) showed that when rats immunised with a large primary larval infection were given a trickle challenge three weeks later a relatively stable challenge worm population was established: they also found that more challenge worms established in rats which had received smaller primary infections.

It is possible that in the final experiment reported here the animals sensitised with 10 L_3 and challenged with 30 L_3 were behaving similarly to those receiving a trickle challenge. This apparent lack of effect on low challenge may be due to the failure of 30 L_3 to reach the threshold level necessary to initiate a significant immune response.

Smith and Christie (1979) reported a similar finding in experimental Haemochus contortus infection in sheep. They noted that compared with their appropriate controls, vaccinated sheep were less consistently resistant to challenge with 500 larvae than

10000 larvae. They suggested that this was due to the existence of a threshold of antigenic information which has to be exceeded if vaccinated animals are to express immunity to challenge.

SECTION 2

GENERAL CONCLUSIONS AND SUMMARY

The main objectives of this study were as follows:
Firstly, to investigate the possible effect of the removal of a sensitising worm burden on the subsequent immune response to *N. brasiliensis* infection in rats.

Secondly, to assess the immunomodulatory effects of several anthelmintics on immunity to *N. brasiliensis*.

Thirdly, to examine the relative immunogenicity of larval and adult *N. brasiliensis*.

The findings can be summarised as follows:

1) An infection consisting of 10 L_3 conferred strong protection against challenge with 3000 L_3 and the immunity thus conferred was long-lasting. There was a short delay in the full expression of the immune response when the sensitising worm burdens were removed by the anthelmintics levamisole, thiabendazole and fenbendazole. When levamisole was used this delay was less in evidence.
2) Levamisole treatment appeared to have a marginal immunostimulant effect when used to remove sensitising infections but when given prior to a primary infection the effect was inconsistent.
3) Gastric intubation was found to be a convenient and rapid non-surgical method of transplanting adult *N. brasiliensis* and 20 adult worms thus transferred conferred a significant degree of protection against challenge with 600 similarly transferred adult worms. However, the degree of immunity was poor compared with that stimulated by 10 L_3 to subsequent challenge with 3000 L_3.

4) No immunomodulatory effect was observed when transplanted adult worms were removed by levamisole a week before adult worm challenge.

5) Ten larvae stimulated a strong degree of protection against challenge even when the sensitising larval infections were terminated after 24 hours.

6) Adult worms stimulated an effective immune response against either larval or adult worm challenge. However, large numbers of adult worms were required to produce the same degree of protection as infection with 10 L_3.

7) The immunity conferred by either 10 L_3 or 250 adult worms was capable of protecting against a challenge of 300 L_3 but when challenge was reduced to 30 L_3 rats immunised with larvae responded poorly while those sensitised with adult worms showed a significant immune response.

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APPENDIX A

Appendix A

Haemonchus contortus infection in sheep

Haemonchus contortus is found in the abomasum of sheep and goats in many parts of the world, most frequently in tropical and sub-tropical areas. Due to the characteristic appearance of the female worm with white ovaries spiralled around the red intestines *H. contortus* is often called the 'barber's pole' worm.

The genus *Haemonchus* was first founded by Cobb (1898) for the species *Strongylus contortus* described by Rudolphi (1803). Later many species have been added to this genus. The *Haemonchus* species found in the abomasum of cattle is called *Haemonchus placei*. The species differentiation between the ovine and bovine isolates was based on host specificity and certain morphological differences of male and female worms recovered from sheep and cattle (Roberts, Turner and McKevitt, 1954; Bremner, 1955). However, Gibbons (1979) states that *H. placei* cannot be differentiated from *H. contortus* on the basis of reliable morphological characters and that the biological differences between *H. placei* and *H. contortus* are not any other than those between strains of the same species.

The life history of *H. contortus* was first worked out experimentally by Ransom (1906) and a brief description of the life cycle is as follows.

Like most *Trichostrongyloidea*, *H. contortus* has a direct life cycle. Eggs are voided in the faeces of infected animals and infective third stage larvae (L_3) develop from these eggs after passing through two free living larval stages (L_1 and L_2) and undergoing two moults. The time taken for the development to the infective larval stage from the egg depends on the environmental temperature and moisture. Under ideal conditions the infective stage is reached

within five to seven days. The infective larvae can withstand extremes of environmental conditions like prolonged drought (Allonby, 1974) and low temperatures for several months (Rose, 1965).

Infection occurs by ingestion of infective larvae. Exsheathment occurs in the rumen and in about two to three days after ingestion larvae undergo the third moult in the abomasum to become fourth stage larvae (L_4). These L_4 develop in the abomasal mucosa but rarely enter the gastric glands (Malczewski, 1971). Further development may be arrested at this stage for long periods, depending on a number of factors including the strain of the parasite, the stimulus received by preparasitic stages, the age and the degree of acquired immunity of the host (Silverman and Patterson, 1960; Dineen, Donald, Wagland and Offner, 1965; Blitz and Gibbs, 1972a,b; Connan, 1971, 1975). The developing L_4 emerge from the mucosa around day 6, grow and moult to become L_5 at about the tenth day after ingestion and reach the mature adult stage on day 15. Eggs first appear in the faeces usually between days 18-24 after infection. In very young lambs the development of all the parasitic stages is particularly rapid and eggs are often produced by day 16 (Silverman and Patterson, 1960). A single mature female worm is capable of producing 10,000 eggs per day for several months in succession (Gordon, 1948). Both larval and adult stages suck blood from the abomasal mucosa (Brambell, Charleston and Tothill, 1964) and in heavy infections in sheep blood loss may be in the region of 200-600 ml/day causing severe anaemia (Allonby and Dargie, 1973).

Appendix A - Experiment 1

Individual PCV % from four groups of lambs recorded twice weekly: Groups I, II and III were immunised with two doses of γ - irradiated H. contortus L_3 and challenged with 10,000 L_3. Groups I and II received anthelmintic treatment three weeks after each vaccination. Group IV received only the challenge infection.

Date	Day	Group I					Group II				
		W6	W7	W8	W9	W10	LY26	LY27	LY28	LY29	LY36
6/12/79	0	38	34	41	34	36	34	35	41	34	39
11/12/79	5	41	32	40	33	38	36	33	39	33	37
14/12/79	8	39	36	42	35	37	35	29	39	34	41
18/12/79	12	34	33	33	29	37	32	29	37	34	40
21/12/79	15	35	33	34	31	35	33	28	36	33	38
24/12/79	18	33	30	34	29	34	31	28	33	31	36
28/12/79	22	34	30	34	28	35	32	28	36	32	35
31/12/79	25	34	29	35	29	33	31	26	36	32	35
3/1/80	28	35	28	34	30	35	31	30	36	32	35
8/1/80	33	35	28	35	29	NS	34	31	39	32	35
11/1/80	36	35	31	35	29	33	33	29	35	31	35
15/1/80	40	35	31	35	28	34	33	31	36	33	37
18/1/80	43	34	31	36	28	34	32	29	35	32	36
22/1/80	47	34	30	33	27	32	32	29	36	29	35
25/1/80	50	33	28	34	25	32	31	28	34	29	34
29/1/80	54	33	28	34	25	33	31	28	35	29	34
1/2/80	57	34	30	33	24	34	32	30	35	30	34
5/2/80	61	34	29	33	27	33	31	30	34	29	34
8/2/80	64	32	29	34	28	33	30	29	34	29	34
12/2/80	68	32	29	32	28	33	31	29	34	29	35
15/2/80	71	29	27	29	23	31	31	29	35	29	34
19/2/80	75	23	24	24	19	30	30	26	35	25	34
22/2/80	78	21	23	21	18	30	31	25	35	24	33
28/2/80	84	20	20	21	17	28	29	24	31	21	31

Contd.

Date	Day	Group III					Group IV					
		LB11	LB12	LB13	LB14	LB15	OR6	OR7	OR8	OR9	OR10	OR11
6/12/79	0	38	37	39	35	40	38	36	35	36	37	36
11/12/79	5	38	36	38	33	40	38	34	36	34	36	35
14/12/79	8	38	36	36	30	43	40	34	36	34	39	37
18/12/79	12	35	35	36	29	37	35	35	36	37	37	33
21/12/79	15	36	35	35	29	37	33	35	36	35	38	34
24/12/79	18	31	35	35	31	37	36	33	32	36	37	36
28/12/79	22	34	32	32	29	36	34	36	30	36	37	34
31/12/79	25	34	34	33	28	35	34	33	30	33	37	32
3/1/80	28	34	31	33	30	36	35	32	30	34	36	32
8/1/80	33	34	30	35	30	36	32	29	33	32	NS	31
11/1/80	36	34	31	34	31	33	32	29	34	35	34	31
15/1/80	40	34	30	35	31	31	30	28	33	33	33	31
18/1/80	43	33	31	34	29	30	30	29	33	33	33	34
22/1/80	47	33	31	34	30	28	30	28	33	33	34	33
25/1/80	50	33	30	34	29	28	32	27	33	33	33	32
29/1/80	54	33	32	34	30	29	32	28	34	34	33	32
1/2/80	57	33	33	32	30	30	31	28	33	33	32	31
5/2/80	61	33	32	33	30	32	31	30	33	33	32	31
8/2/80	64	33	33	33	31	33	30	27	31	32	32	30
12/2/80	68	33	32	33	30	33	26	23	25	28	32	28
15/2/80	71	34	32	34	29	29	22	22	22	22	32	21
19/2/80	75	34	31	28	27	25	20	20	17	21	30	19
22/2/80	78	34	32	25	24	25	18	22	16	20	30	19
28/2/80	84	33	31	24	24	23	19	21	16	17	27	18

Appendix A - Experiment 2

Individual PCV % from four groups of lambs recorded at twice weekly intervals: Groups V, VI and VII received two doses of 10,000 irradiated L_3 and a dose of 10,000 normal H. contortus L_3 all four groups were challenged with 10,000 normal L_3 four weeks after the last infection. Groups V and VI received anthelmintic treatment one week before the challenge infection.

Date	Day	Group V					Group VI				
		Y58	Y82	Y83	Y84	Y85	P1	P2	P3	P41	P5
6/12/79	0	34	41	41	35	38	39	34	35	41	37
11/12/79	5	35	39	38	33	37	37	35	32	39	32
14/12/79	8	35	41	43	37	38	37	35	38	42	36
18/12/79	12	34	36	39	30	38	36	35	34	34	36
21/12/79	15	30	38	36	28	36	35	35	30	36	28
24/12/79	18	33	32	34	29	31	38	34	30	35	27
28/12/79	22	34	33	35	29	32	36	34	34	36	30
31/12/79	25	32	34	36	28	32	32	33	32	35	32
3/1/80	28	32	33	35	28	32	35	33	33	36	30
8/1/80	33	32	34	35	30	36	35	35	34	36	29
11/1/80	36	34	35	38	30	36	36	35	32	35	31
15/1/80	40	33	38	39	32	36	36	35	32	34	32
18/1/80	43	31	37	37	30	36	35	34	32	33	33
22/1/80	47	32	36	37	30	35	37	34	31	34	31
25/1/80	50	32	37	37	30	35	35	32	29	32	30
29/1/80	54	34	36	37	30	35	35	32	29	32	28
1/2/80	57	34	37	36	30	35	34	33	30	33	30
5/2/80	61	34	37	36	31	35	34	33	31	33	29
8/2/80	64	33	37	37	32	35	33	32	30	32	29
12/2/80	68	33	37	38	29	36	31	33	30	33	27
15/2/80	71	33	37	39	29	36	30	33	30	30	26
19/2/80	75	33	35	36	29	31	28	30	29	26	24
22/2/80	78	34	36	37	31	30	31	32	29	26	25
26/2/80	82	34	36	37	31	31	30	31	29	30	26
29/2/80	85	36	37	35	31	33	33	31	33	32	28
4/3/80	89	35	37	36	31	32	32	32	33	32	29
7/3/80	92	35	37	40	31	35	33	34	32	33	25
11/3/80	96	36	35	35	31	35	32	32	32	33	26
13/3/80	98	32	34	35	29	32	29	33	27	31	26
17/3/80	102	32	34	35	29	31	28	34	23	30	21
20/3/80	105	35	35	36	29	29	27	32	24	28	19
27/3/80	112	32	33	36	29	26	30	34	28	27	23

Contd.

Date	Day	Group VII					Group VIII					
		R6	R7	R8	R9	R10	DB6	DB7	DB8	DB9	DB10	DB11
6/12/79	0	39	39	39	38	35	36	41	39	33	40	34
11/12/79	5	35	35	38	38	32	38	39	37	37	41	36
14/12/79	8	38	38	42	42	36	36	40	38	34	44	39
18/12/79	12	34	34	38	36	35	34	35	39	35	36	31
21/12/79	15	34	31	34	35	32	33	35	36	32	36	32
24/12/79	18	30	32	34	37	34	38	35	36	35	35	38
28/12/79	22	32	30	33	38	31	38	40	39	34	40	35
31/12/79	25	32	32	30	35	31	34	39	NS	32	39	34
3/1/80	28	32	32	32	37	30	36	38	39	32	40	34
8/1/80	33	31	32	32	34	29	34	36	39	32	40	32
11/1/80	36	33	32	33	35	31	33	35	39	32	38	33
15/1/80	40	31	32	32	33	28	35	37	37	33	38	33
18/1/80	43	30	34	33	34	30	35	36	38	32	39	33
22/1/80	47	31	34	34	34	29	35	37	38	33	38	32
25/1/80	50	32	33	35	33	31	35	36	38	34	38	32
29/1/80	54	30	34	33	33	30	36	36	37	33	40	33
1/2/80	57	32	35	34	34	32	35	35	40	33	39	32
5/2/80	61	34	35	34	34	28	35	37	38	32	39	32
8/2/80	64	NS	35	34	33	29	34	37	37	32	38	32
12/2/80	68	NS	34	33	32	28	34	35	35	31	37	32
15/2/80	71	26	33	33	33	28	33	35	35	30	37	31
19/2/80	75	NS	33	32	33	28	35	34	36	31	40	33
22/2/80	78	NS	35	31	33	30	34	34	36	30	39	32
26/2/80	82	Died	35	32	32	30	34	36	39	32	40	33
29/2/80	85	-	36	32	33	31	34	35	37	31	39	34
4/3/80	89	-	34	30	33	28	33	32	35	30	38	32
7/3/80	92	-	34	31	34	39	34	34	34	31	38	28
11/3/80	96	-	31	29	30	30	29	30	34	25	35	26
13/3/80	98	-	32	27	31	28	26	26	34	21	29	20
17/3/80	102	-	32	25	31	28	24	21	32	18	21	16
20/3/80	105	-	30	26	28	29	23	25	35	19	25	19
27/3/80	112	-	32	30	28	25	23	30	37	19	25	24

Appendix A - Experiment 3

Individual PCV % recorded twice weekly from six groups of lambs: Groups I, II and III received 10,000, 40 kr γ - irradiated, 60 kr γ - irradiated and normal Cambridge strain *H. contortus* L_3 respectively. Groups IV, V and VI received 10,000, 40 kr γ - irradiated, 60 kr γ - irradiated and normal Glasgow strain *H. contortus* L_3 respectively.

Date	Day	Group I			Group II			Group III			Group IV			Group V			Group VI		
		W11	W12	W13	P51	P52	P53	LB21	LB22	LB25	R34	R35	R36	LY81	LY82	LY83	FR73	FR74	FR75
9/7/80	0	31	33	28	36	34	28	30	32	35	31	30	28	31	34	30	32	32	34
11/7/80	2	31	34	28	35	34	28	32	31	36	32	31	27	31	35	30	34	33	32
14/7/80	5	32	35	27	35	34	29	31	29	36	31	31	29	31	35	30	31	31	32
17/7/80	8	32	34	29	34	34	27	31	29	36	31	31	29	29	35	30	31	31	32
22/7/80	13	32	34	28	32	35	31	24	26	34	31	32	28	31	35	33	24	29	22
24/7/80	15	30	33	25	31	32	29	23	27	27	30	34	20	31	36	33	16	25	14
28/7/80	19	27	32	22	29	30	28	22	25	23	29	31	23	31	32	30	14	20	13
31/7/80	22	29	32	22	29	30	27	25	23	23	29	30	22	29	32	27	12	18	11
4/8/80	26	35	33	27	32	33	29	28	29	28	32	33	26	32	36	32	14	23	12

Appendix A - Experiment 4

Individual PCV % recorded at twice weekly intervals from four groups of lambs: Groups VII and IX were immunised with two doses of 60 kr γ-irradiated Glasgow and Cambridge strain H. contortus L_3 respectively. Four weeks after the second vaccination Groups VII and VIII received 10,000 normal Glasgow strain L_3 and Groups IX and X received 10,000 normal Cambridge strain L_3.

Date	Day	Group VII				Group VIII			
		R67	R68	R69	R70	DB69	DB70	DB71	DB72
23/9/80	0	36	34	34	30	35	34	34	31
26/9/80	3	35	34	34	30	35	34	33	31
30/9/80	7	37	36	35	30	35	35	32	31
3/10/80	10	36	33	35	31	36	34	32	32
6/10/80	13	35	36	35	31	36	34	31	32
9/10/80	16	33	30	33	29	37	34	31	31
14/10/80	21	34	31	34	31	35	35	31	32
17/10/80	24	33	23	34	29	35	36	32	32
20/10/80	27	32	24	33	27	36	37	32	32
24/10/80	31	33	28	32	25	35	37	34	33
27/10/80	34	34	29	33	25	34	36	33	32
30/10/80	37	34	32	32	26	34	36	34	31
3/11/80	41	34	33	31	27	35	37	33	31
6/11/80	44	35	35	29	27	35	37	34	31
10/11/80	48	33	33	25	26	36	37	35	31
13/11/80	51	34	34	26	28	38	36	36	32
17/11/80	55	34	33	28	28	36	37	36	32
20/11/80	58	32	36	30	28	37	39	36	32
24/11/80	62	29	36	28	28	35	38	33	30
27/11/80	65	32	35	30	28	37	38	34	32
1/12/80	69	33	36	31	25	25	33	32	27
4/12/80	72	31	34	33	23	18	29	30	22
8/12/80	76	26	33	34	17	18	27	28	23
11/12/80	79	27	35	35	24	20	24	29	21
16/12/80	84	29	37	37	25	20	26	28	22

Contd.

Date	Day	Group IX				Group X			
		P77	P78	P79	P80	LY73	LY74	LY75	LY76
23/9/80	0	37	34	35	38	40	35	32	39
26/9/80	3	36	34	34	38	40	35	32	37
30/9/80	7	37	34	30	35	40	35	33	37
3/10/80	10	37	32	31	34	40	35	34	37
6/10/80	13	38	33	31	35	39	35	34	37
9/10/80	16	36	33	31	36	40	35	35	38
14/10/80	21	36	33	32	36	39	35	34	36
17/10/80	24	36	34	32	36	39	36	32	37
20/10/80	27	36	34	30	35	39	36	32	35
24/10/80	31	34	34	28	34	40	35	32	35
27/10/80	34	35	35	26	34	39	35	34	31
30/10/80	37	35	35	28	33	39	35	34	31
3/11/80	41	35	33	29	33	39	35	34	35
6/11/80	44	35	30	29	33	39	35	34	35
10/11/80	48	35	30	31	34	39	35	34	35
13/11/80	51	34	31	32	26	41	35	35	37
17/11/80	55	28	31	33	22	40	34	34	36
20/11/80	58	28	33	33	25	41	34	35	37
24/11/80	62	33	34	34	28	39	34	29	35
27/11/80	65	34	33	33	33	38	35	30	34
1/12/80	69	34	33	32	33	37	34	30	33
4/12/80	72	37	36	30	36	37	32	26	33
8/12/80	76	35	34	26	35	35	31	28	31
11/12/80	79	38	35	27	34	35	31	24	34
16/12/80	84	37	33	24	33	37	23	25	33

Appendix A - Experiment 5

Individual PCV% recorded at weekly intervals from four groups of lambs: Groups XI and XIII were immunised with two doses of 60 kr γ - irradiated Glasgow and Cambridge strain L_3 respectively. Four weeks after the second vaccination Groups XI and XII received 10,000 normal Glasgow strain L_3 and Groups XIII and XIV received 10,000 normal Cambridge strain L_3 respectively.

Date	Day	Group XI				Group XII				Group XIII				Group XIV			
		W4	W8	Y11	B24	W5	W7	W10	Y19	W9	Y14	Y18	B22	Y13	B21	B23	B25
23/9/80	0	37	42	40	40	36	38	36	38	43	40	43	41	33	38	37	45
30/9/80	7	37	37	39	36	33	37	37	37	36	36	41	41	35	38	35	42
7/10/80	14	36	36	38	39	35	39	33	36	37	36	39	42	33	37	36	44
14/10/80	21	34	37	39	38	36	40	35	38	35	37	40	39	35	38	36	43
21/10/80	28	35	38	38	34	35	36	37	36	38	34	34	40	33	37	38	42
27/10/80	34	36	37	40	37	33	37	38	37	38	36	35	42	36	38	37	40
11/11/80	49	30	38	35	36	33	38	37	36	32	36	37	32	34	38	36	39
18/11/80	56	30	36	35	35	36	37	37	38	32	35	39	NS	37	37	39	35
25/11/80	63	31	37	39	36	36	38	39	33	35	37	40	37	37	39	39	33
2/12/80	70	30	37	29	33	33	31	37	24	35	37	37	34	36	37	34	27
9/12/80	77	20	30	31	34	33	30	23	23	40	28	36	35	33	32	26	26

Appendix A - Experiment 6

Individual PCV % recorded twice weekly from two groups of lambs: Group I was immunised with two doses of 10,000 normal Glasgow strain *H. contortus* L_3. Three weeks after the second infection Group I and II received anthelmintic treatment and one week later both groups were given 10,000 normal L_3.

Date	Day	Group I			Group II		
		R41	R42	R43	G47	G56	G57
17/2/81	0	36	36	36	38	35	30
24/2/81	7	33	35	33	37	35	30
27/2/81	10	34	34	34	39	35	30
3/3/81	14	31	34	30	37	34	30
6/3/81	17	30	34	29	36	34	30
10/3/81	21	26	30	30	37	34	27
13/3/81	24	24	32	28	36	34	28
17/3/81	28	23	29	28	38	34	27
24/3/81	35	26	30	28	38	34	28
27/3/81	38	30	32	28	37	35	29
31/3/81	42	25	30	27	37	33	29
7/4/81	49	25	32	27	37	30	29
14/4/81	56	28	29	27	36	30	29
21/4/81	63	27	30	29	35	27	23
28/4/81	70	28	30	27	31	26	20
5/5/81	77	24	28	30	27	23	20
12/5/81	84	22	29	32	26	20	19

Appendix A - Experiment 7

Individual PCV % recorded at weekly intervals from six groups of lambs: Groups III and IV were immunised with two doses of Glasgow strain H. contortus L_3 irradiated at 40 kr with γ - rays and X - rays respectively. Groups VI and VII were immunised with two doses of MSD strain H. contortus L_3 irradiated at 40 kr with γ - rays and X - rays respectively. Four weeks after the second vaccination Groups III, IV and V were given 10,000 normal Glasgow strain L_3 while Groups VI, VII and VIII were given 10,000 normal MSD L_3.

Date	Day	Group III				Group IV				Group V			
		DY77	DY78	DY79	DY80	P56	P57	P58	P59	LY58	LY59	LY60	LY65
3/3/81	0	31	37	35	38	38	36	34	34	38	40	38	33
10/3/81	7	31	34	33	40	37	36	33	34	35	39	37	30
17/3/81	14	33	35	35	39	35	37	34	34	37	40	40	36
24/3/81	21	33	35	35	39	35	38	33	32	36	40	39	34
31/3/81	28	32	28	35	39	34	37	33	32	35	38	39	34
7/4/81	35	29	25	35	39	33	37	34	31	36	38	40	34
14/4/81	42	28	29	35	35	32	34	32	31	33	37	38	34
21/4/81	49	28	30	34	34	33	33	33	31	33	37	37	32
28/4/81	56	31	33	29	36	31	31	34	29	32	37	36	32
5/5/81	63	31	31	29	35	31	32	34	32	30	36	35	30
12/5/81	70	31	33	28	36	32	28	32	30	26	32	32	28
19/5/81	77	34	32	32	35	32	32	35	30	22	25	28	24
26/5/81	84	35	33	33	33	29	31	33	30	22	25	Died	22

Contd.

Date	Day	Group VI				Group VII				Group VIII			
		OR12	OR13	OR14	OR15	DB12	DB14	DB21	DB22	R37	R38	R39	R40
3/3/81	0	28	33	35	34	31	35	40	38	33	34	35	36
10/3/81	7	27	34	35	32	32	33	40	36	32	32	34	37
17/3/81	14	28	30	34	32	31	29	40	36	34	32	35	36
24/3/81	21	28	30	31	32	32	33	40	32	33	33	34	37
31/3/81	28	27	29	30	32	27	32	38	35	34	33	33	37
7/4/81	35	27	33	30	33	29	33	40	34	35	33	34	37
14/4/81	42	26	33	32	32	28	34	37	35	34	30	30	35
21/4/81	49	26	34	33	32	24	30	36	37	33	30	28	33
28/4/81	56	28	36	30	33	23	34	35	36	33	29	28	28
5/5/81	63	27	35	30	30	23	34	36	37	31	30	28	27
12/5/81	70	28	34	33	30	22	34	36	37	28	30	20	28
19/5/81	77	27	34	31	32	29	28	34	37	21	26	16	26
26/5/81	84	25	27	30	33	28	26	28	37	21	23	16	26

Appendix A - Experiment 8

Individual PCV % recorded weekly from five groups of lambs: Groups IX and X were immunised with two doses of Glasgow strain H. contortus L_3 γ - irradiated at 40 kr and 60 kr respectively. Group XII was immunised with two doses of MSD strain H. contortus L_3 γ - irradiated at 40 kr. Four weeks after the second vaccination Groups IX, X and XI received 10,000 normal Glasgow strain H. contortus L_3 while Groups XII and XIII received 10,000 normal MSD L_3.

Date	Day	Group IX					Group X					Group XI				
		R1	R2	R3	R4	R5	P11	P12	P13	P15	P16	FR11	FR12	FR13	FR14	FR15
1/12/81	-8	41	40	44	33	37	42	43	40	40	42	43	41	44	42	41
9/12/81	0	40	35	40	33	34	37	35	35	38	36	42	37	40	38	38
16/12/81	7	40	38	43	37	36	41	40	38	39	38	41	39	40	41	43
23/12/81	14	37	39	43	34	36	39	35	39	36	38	43	41	42	40	41
30/12/81	21	37	33	42	33	34	41	34	35	38	35	43	40	42	39	38
6/1/82	28	35	35	39	32	32	38	35	36	39	35	38	36	40	36	35
13/1/82	35	36	35	38	33	38	40	38	38	38	38	40	40	38	38	36
20/1/82	42	34	34	35	32	32	39	37	34	33	34	38	36	34	38	34
27/1/82	49	32	32	34	32	31	37	33	33	36	32	38	34	37	35	34
3/2/82	56	36	34	38	33	32	35	35	33	34	34	38	36	38	33	35
17/2/82	70	32	34	33	31	28	36	35	30	35	31	34	31	37	32	32
24/2/82	77	37	31	31	28	30	34	33	32	35	32	28	29	31	31	29
1/3/82	82	34	30	35	28	28	36	36	31	32	30	29	33	34	32	30

Contd.

Date	Day	Group XII					Group XIII				
		G11	G12	G13	G14	G15	DY1	DY2	DY3	DY4	DY5
1/12/81	-8	43	45	41	42	43	41	39	43	38	37
9/12/81	0	44	40	37	38	39	39	39	41	36	34
16/12/81	7	44	41	36	39	40	39	38	38	35	41
23/12/81	14	42	41	35	38	45	41	37	41	37	42
30/12/81	21	43	41	36	38	39	40	38	38	36	39
6/1/82	28	41	37	35	36	Died	39	36	41	34	36
13/1/82	35	38	40	Died	36	-	37	38	36	33	38
20/1/82	42	36	38	-	34	-	38	32	36	37	36
27/1/82	49	32	36	-	32	-	37	33	38	32	35
3/2/82	56	35	40	-	34	-	36	34	35	31	37
17/2/82	70	34	37	-	35	-	28	25	38	24	33
24/2/82	77	34	40	-	32	-	25	24	33	26	28
1/3/82	82	31	36	-	35	-	25	22	32	28	29

Appendix A - Experiment 1

Individual and mean Hb % recorded at weekly intervals from four groups of lambs: Groups I, II and III were immunised with two doses of irradiated H. contortus L_3, Groups I and II received anthelmintic treatment three weeks after each vaccination. Four weeks after the second vaccination all four groups were given 10,000 normal L_3.

Date	Days After Infection	Group I						Group II					
		W6	W7	W8	W9	W10	Mean	LY26	LY27	LY28	LY29	LY36	Mean
18/12/79	12	12.3	11.3	11.1	10.0	11.3	11.2	11.7	9.7	12.4	12.0	12.9	11.7
24/12/79	18	11.2	10.2	11.2	9.8	11.5	10.8	9.9	9.0	11.2	10.9	12.3	10.7
31/12/79	25	12.1	10.1	11.8	10.1	11.1	11.0	10.6	9.2	12.4	11.5	11.5	11.0
8/1/80	33	12.1	10.3	11.5	10.1	NS	11.0	12.4	10.8	13.4	11.5	12.3	12.1
15/1/80	40	12.4	10.8	12.1	9.8	11.9	11.4	11.1	11.1	12.8	11.5	12.4	11.8
22/1/80	47	12.1	10.1	11.1	9.8	11.8	11.0	11.5	10.8	12.8	11.1	12.3	11.7
29/1/80	54	11.5	9.8	11.5	9.2	11.9	10.8	10.6	10.1	11.9	10.6	11.1	10.9
5/2/80	61	12.3	10.6	11.8	10.1	12.4	11.4	10.8	10.5	12.1	10.8	11.8	11.2
12/2/80	68	11.1	10.2	11.6	9.5	12.4	11.0	11.5	10.2	12.4	11.0	12.8	11.6
19/2/80	75	8.1	10.0	8.1	6.5	10.7	8.7	10.7	10.0	12.6	9.2	11.5	10.8
26/2/80	82	6.5	6.6	7.0	5.6	9.5	7.0	10.0	8.4	10.5	7.4	11.1	9.5

Contd.

Date	Days After Infection	Group III						Group IV						
		LB11	LB12	LB13	LB14	LB15	Mean	OR6	OR7	OR8	OR9	OR10	OR11	Mean
18/12/79	12	12.0	12.3	12.6	10.0	11.5	11.7	12.3	12.3	9.4	11.3	13.0	12.0	11.7
24/12/79	18	10.9	11.2	11.2	9.8	11.2	10.9	11.5	11.1	9.9	10.2	12.2	11.2	11.0
31/12/79	25	11.8	10.5	11.5	10.0	11.5	11.1	11.3	11.8	10.5	11.5	12.1	11.5	11.5
8/1/80	33	11.5	10.8	12.3	10.8	11.3	11.3	11.5	10.1	11.1	11.0	N.S.	11.0	10.9
15/1/80	40	10.8	11.0	12.1	10.8	10.1	11.0	10.6	9.6	10.5	11.1	10.5	10.8	10.5
22/1/80	47	11.8	11.1	11.5	10.3	9.5	10.8	10.8	10.1	10.8	10.5	11.3	11.0	10.8
29/1/80	54	11.5	11.5	12.1	10.6	9.8	11.1	10.8	9.2	11.8	11.1	11.1	9.7	10.6
5/2/80	61	11.6	11.8	12.1	11.0	11.1	11.5	11.5	10.3	11.8	11.0	11.9	11.5	11.3
12/2/80	68	10.8	11.5	9.3	12.4	10.8	11.0	9.8	9.3	11.5	9.7	12.1	11.5	10.7
19/2/80	75	11.3	10.7	9.4	9.4	7.4	9.6	6.6	7.4	5.8	7.1	10.7	7.1	7.5
26/2/80	82	11.0	10.5	7.9	8.5	7.9	9.2	6.2	8.1	4.9	5.6	9.5	6.2	6.8

Appendix A - Experiment 2

Individual and mean Hb % recorded at weekly intervals from four groups of lambs: Groups V, VI and VII were given two doses of 10,000 irradiated and a dose of normal H. contortus L_3 with a four week interval between each dose. All four groups were challenged with 10,000 L_3 four weeks after the last infection. Groups V and VI received anthelmintic treatment one week before challenge.

Date	Days Post Infection	Group V						Group VI					
		Y58	Y82	Y83	Y84	Y85	Mean	P1	P2	P3	P41	P5	Mean
18/12/79	12	11.5	10.9	12.9	10.7	12.0	11.6	12.6	12.3	11.7	12.3	9.4	11.7
24/12/79	18	10.9	10.6	10.9	9.8	10.0	10.4	11.8	11.2	9.9	11.3	8.9	10.6
31/12/79	25	11.1	11.5	12.1	10.1	10.8	11.1	10.5	11.5	11.1	11.8	11.2	11.2
8/1/80	33	10.3	11.9	12.1	11.1	11.8	11.4	11.5	12.1	11.6	12.4	9.8	11.5
15/1/80	40	11.1	12.9	13.1	12.1	12.4	12.3	12.4	11.8	11.5	12.1	11.6	11.9
22/1/80	47	11.0	13.1	12.8	11.0	12.4	12.1	12.8	12.1	11.1	11.8	11.1	11.8
29/1/80	54	11.8	12.8	12.8	11.5	12.4	12.3	11.5	11.5	10.5	11.6	10.0	11.0
5/2/80	61	12.8	13.7	13.9	11.5	12.8	12.9	11.6	11.8	11.9	11.6	10.0	11.4
12/2/80	68	11.8	12.4	12.3	9.8	12.4	11.7	11.0	11.6	11.5	11.3	9.8	11.0
19/2/80	75	11.3	11.8	11.6	10.8	10.4	11.2	9.9	10.0	10.5	8.6	8.6	9.5
26/2/80	82	10.8	12.8	13.4	10.6	11.0	11.7	10.8	11.1	10.8	10.2	8.8	10.3
4/3/80	89	11.3	12.0	12.3	11.0	11.0	11.5	10.7	11.3	10.4	11.7	9.7	10.8
11/3/80	96	11.5	12.2	12.2	10.9	11.0	11.6	10.3	10.6	9.3	11.2	9.8	10.2
17/3/80	102	11.2	12.0	12.3	9.7	10.5	11.1	9.6	11.8	8.1	9.1	7.1	9.1
25/3/80	110	10.6	11.8	12.2	9.6	9.3	10.7	9.6	11.2	9.6	9.3	7.0	9.3

Contd.

Date	Days Post Infection	Group VII						Group VIII						
		R6	R7	R8	R9	R10	Mean	DB6	DB7	DB8	DB9	DB10	DB11	Mean
18/12/79	12	12.4	11.6	11.9	12.6	11.7	12.0	11.3	11.8	12.0	11.6	12.3	10.4	11.6
24/12/79	18	12.2	10.5	11.2	10.2	11.2	11.1	11.5	11.5	11.5	11.2	12.5	12.2	11.7
31/12/79	25	11.1	10.8	10.1	12.1	10.8	11.0	11.8	13.1	NS	11.1	13.1	12.3	12.3
8/1/80	33	11.9	11.6	11.0	10.8	10.0	11.1	11.5	12.4	13.1	10.8	13.4	10.8	12.0
15/1/80	40	10.5	11.8	11.1	11.9	9.8	11.0	11.9	13.0	12.4	10.9	12.4	11.9	12.1
22/1/80	47	10.8	11.8	11.8	12.1	10.3	11.4	11.5	11.9	12.1	10.4	11.8	10.6	11.4
29/1/80	54	10.8	11.5	10.8	11.6	10.8	11.1	11.1	11.3	12.1	11.0	14.1	11.0	11.8
5/2/80	61	12.6	12.6	11.8	11.3	10.2	11.7	12.1	12.4	11.8	10.5	12.4	11.3	11.8
12/2/80	68	8.2	12.3	10.8	11.1	10.8	10.6	11.3	11.5	11.8	9.8	12.4	11.1	11.3
19/2/80	75	7.9	12.5	10.7	12.1	10.4	10.7	12.3	11.3	11.8	10.4	13.4	11.3	11.8
26/2/80	82	7.7	11.8	10.6	11.5	10.8	10.5	11.5	11.8	12.4	11.1	13.0	11.0	11.8
4/3/80	89	Died	11.3	10.0	11.3	9.7	10.6	11.0	10.5	11.7	10.4	12.3	11.0	11.2
11/3/80	96	-	11.2	9.9	10.6	9.9	10.4	9.9	9.6	11.2	9.4	12.2	9.3	10.3
17/3/80	102	-	11.0	8.4	10.5	9.4	9.8	7.9	7.1	11.3	6.2	8.1	6.1	7.8
25/3/80	110	-	10.9	9.6	9.9	8.3	9.7	7.7	9.6	12.8	5.9	7.4	8.0	8.6

Appendix A - Experiment 1

Individual and mean RBC (x 10^{12}/lit) counts recorded at weekly intervals from four groups of lambs: Groups I, II and III were immunised with two doses of irradiated H. contortus L_3. Groups I and II received anthelmintic treatment three weeks after each vaccination. Four weeks after the second vaccination all four groups were given 10,000 normal L_3.

Date	Days After Infection	Group I						Group II					
		W6	W7	W8	W9	W10	Mean	LY26	LY27	LY28	LY29	LY36	Mean
18/12/79	12	11.6	10.4	10.2	9.2	10.2	10.3	10.8	8.2	11.2	10.4	11.4	10.4
24/12/79	18	11.0	9.6	10.6	8.8	10.6	10.1	9.8	8.2	10.2	10.0	11.8	10.0
31/12/79	25	11.8	9.2	8.0	9.2	9.6	9.6	9.8	8.2	11.2	10.4	11.0	10.1
8/1/80	33	11.0	8.8	9.4	8.6	N.S.	9.5	11.4	8.6	12.0	9.2	11.2	10.5
15/1/80	40	11.0	9.8	10.8	8.4	10.4	10.1	10.8	9.2	11.8	10.0	11.8	10.7
22/1/80	47	11.4	9.0	10.0	8.4	9.6	9.7	10.2	8.6	11.6	9.6	11.2	10.2
29/1/80	54	11.4	9.2	10.0	7.8	10.0	9.7	10.0	8.6	10.8	8.8	10.4	9.7
5/2/80	61	11.6	9.2	10.4	8.0	12.2	10.3	9.6	8.8	11.2	8.6	11.0	9.8
12/2/80	68	10.6	9.2	10.2	7.8	10.0	9.6	10.6	8.6	10.8	9.0	11.2	10.0
19/2/80	75	7.4	7.8	6.4	5.6	9.2	7.3	9.8	7.2	11.8	7.4	10.8	9.4
26/2/80	82	5.0	6.2	5.4	4.6	8.4	5.9	9.4	7.0	10.2	6.2	10.8	8.7

Contd.

Date	Days After Infection	Group III						Group IV						
		LB11	LB12	LB13	LB14	LB15	Mean	OR6	OR7	OR8	OR9	OR10	OR11	Mean
18/12/79	12	11.2	11.0	11.2	8.8	10.2	10.5	10.8	11.2	8.6	10.2	11.4	10.2	10.4
24/12/79	18	10.8	11.2	10.6	9.6	10.4	10.5	10.6	10.2	9.4	9.7	11.4	11.2	10.4
31/12/79	25	10.4	9.6	10.0	8.6	9.8	9.7	10.0	11.2	9.0	10.0	11.0	10.0	10.2
8/1/80	33	10.8	9.0	10.4	9.2	9.4	9.8	10.6	8.6	10.0	9.6	N.S.	9.8	9.7
15/1/80	40	10.6	10.2	10.8	9.2	8.6	9.9	9.8	9.0	9.4	9.8	9.8	9.2	9.5
22/1/80	47	10.6	9.6	10.0	9.0	8.0	9.4	9.4	8.4	10.0	9.4	9.8	9.2	9.4
29/1/80	54	10.6	10.6	11.0	10.4	8.2	10.2	9.6	7.8	10.0	9.8	9.6	8.6	9.2
5/2/80	61	11.0	10.4	10.8	9.6	8.6	10.1	9.8	9.2	10.8	9.4	10.2	8.4	9.6
12/2/80	68	11.2	10.2	10.0	9.6	8.8	10.0	8.6	7.2	8.4	8.0	10.4	8.4	8.5
19/2/80	75	11.2	10.2	8.8	8.2	6.2	8.9	6.2	6.2	5.4	5.6	9.2	6.0	6.4
26/2/80	82	11.0	10.0	6.2	7.8	5.4	8.1	5.0	6.8	4.0	4.0	9.0	5.4	5.7

Appendix A - Experiment 2

Individual and mean RBC (x 10^{12} lit) counts recorded at weekly intervals from four groups of lambs: Groups V, VI and VII received two doses of 10,000 irradiated and a dose of normal H. contortus L_3 with a four week interval between each dose. All four groups were challenged with 10,000 L_3 four weeks after the last infection. Groups V and VI received anthelmintic treatment one week before challenge.

Date	Days After Infection	Group V						Group VI					
		Y58	Y82	Y83	Y84	Y85	Mean	P1	P2	P3	P41	P5	Mean
18/12/79	12	10.2	9.2	12.2	12.6	11.8	11.2	10.6	11.4	10.4	11.2	8.8	10.5
24/12/79	18	10.6	9.4	10.4	9.0	9.6	9.8	10.4	11.2	8.8	10.2	8.4	9.8
31/12/79	25	10.0	9.6	10.6	8.5	9.8	9.7	9.2	10.4	9.8	10.8	9.4	9.9
8/1/80	33	9.4	9.8	9.9	9.6	9.6	9.7	9.8	10.4	10.6	10.4	8.6	10.0
15/1/80	40	10.4	11.0	10.4	10.6	11.0	10.7	9.8	11.4	10.4	11.0	10.2	10.6
22/1/80	47	9.6	11.0	10.6	9.8	10.6	10.3	10.8	11.4	10.4	10.4	10.4	10.7
29/1/80	54	11.0	11.0	11.6	10.4	10.8	11.0	9.4	11.6	9.2	10.8	8.8	10.0
5/2/80	61	11.0	11.8	11.8	9.4	10.8	11.0	10.4	10.8	10.6	10.6	9.2	10.3
12/2/80	68	10.4	10.8	11.2	9.8	10.8	10.6	9.2	11.0	9.6	10.0	8.8	9.7
19/2/80	75	9.8	10.2	10.4	9.6	9.2	9.8	9.0	9.6	9.8	8.0	7.0	8.7
26/2/80	82	10.6	10.4	12.0	9.4	9.6	10.4	9.6	11.0	9.8	8.8	7.6	9.4
4/3/80	89	10.8	10.6	11.6	9.8	9.8	10.5	9.6	10.8	10.0	9.6	9.0	9.8
11/3/80	96	11.0	10.6	11.4	10.2	10.4	10.7	8.6	10.8	10.0	9.8	7.8	9.4
17/3/80	102	10.4	10.2	11.0	9.4	10.0	10.2	8.4	11.0	7.4	7.8	6.6	8.2
25/3/80	110	10.0	10.2	11.0	9.6	8.4	9.8	8.2	10.6	8.2	6.4	6.6	8.0

Contd.

Date	Days After Infection	Group VII						Group VIII						
		R6	R7	R8	R9	R10	Mean	DB6	DB7	DB8	DB9	DB10	DB11	Mean
18/12/79	12	11.2	10.4	10.8	11.2	10.0	10.7	9.8	11.0	11.4	10.4	11.0	9.4	10.5
24/12/79	18	10.8	9.4	10.8	9.2	10.2	10.1	11.0	10.2	10.8	11.0	11.0	10.8	10.8
31/12/79	25	9.6	8.8	9.2	10.2	9.4	9.4	10.8	11.8	N.S.	10.8	11.0	10.3	10.9
8/1/80	33	9.4	9.0	9.6	9.6	8.2	9.2	9.8	10.6	12.0	9.4	10.8	9.6	10.4
15/1/80	40	9.4	8.8	9.6	10.2	8.6	9.3	10.8	11.8	11.2	10.4	10.8	11.0	11.0
22/1/80	47	9.4	10.2	10.6	10.4	8.8	9.9	10.2	10.6	11.2	10.0	10.2	9.6	10.3
29/1/80	54	9.8	9.6	10.8	10.0	9.8	10.0	10.0	10.2	11.2	10.4	12.2	9.6	10.6
5/2/80	61	10.4	10.4	10.0	9.2	8.6	9.7	11.0	11.0	11.2	9.2	11.4	9.4	10.5
12/2/80	68	6.8	9.8	10.6	8.8	9.0	9.0	9.6	10.0	11.2	9.0	11.0	9.4	10.0
19/2/80	75	6.6	10.6	9.6	10.0	8.6	9.1	11.2	10.0	11.2	9.6	12.0	10.0	10.7
26/2/80	82	6.6	9.8	9.8	9.4	9.4	9.0	10.4	10.8	12.0	10.2	12.0	9.8	10.9
4/3/80	89	Died	8.8	9.6	9.8	8.6	9.2	9.8	10.2	10.8	9.6	11.0	9.6	10.2
11/3/80	96	-	9.6	9.2	8.6	8.8	9.1	9.6	9.2	10.6	8.2	10.8	8.4	9.5
17/3/80	102	-	9.4	8.2	9.0	8.4	8.8	6.6	6.6	10.2	4.0	6.4	5.4	6.5
25/3/80	110	-	9.6	9.0	8.4	7.6	8.7	5.8	8.2	11.4	4.8	5.8	6.8	7.1

Appendix A - Experiment 1

Individual plasma pepsinogen levels (m.u. of tyrosine) recorded at weekly intervals from four groups of lambs: Groups I, II and III were immunised with two doses of γ- irradiated *H. contortus* L_3 and challenged with 10,000 L_3. Groups I and II received anthelmintic treatment three weeks after each vaccination Group IV received only the challenge infection.

Date	Days	Group I					Group II				
		W6	W7	W8	W9	W10	LY26	LY27	LY28	LY29	LY36
6/12/79	0	596	359	464	388	403	643	506	536	359	657
14/12/79	8	557	720	542	582	637	2237	705	723	718	829
21/12/79	15	669	599	490	640	668	1221	902	891	863	1082
28/12/79	22	692	424	518	544	491	988	692	947	571	1028
3/1/80	28	601	348	454	414	441	696	576	628	455	655
11/1/80	36	1292	1074	979	1469	1374	2533	1347	2274	1728	2274
18/1/80	43	1221	878	760	876	921	2340	1234	2118	1730	2195
25/1/80	50	690	595	692	719	609	1761	802	1413	913	1706
1/2/80	57	587	436	585	509	441	1286	700	817	489	1000
8/2/80	64	1091	1284	1138	1519	1013	4080	1978	2847	1345	5648
15/2/80	71	1363	988	858	1392	827	3162	1089	2554	1189	2379
22/2/80	78	770	831	655	1049	801	1585	904	1539	946	1669
26/2/80	82	583	516	583	886	675	1230	756	1496	755	1469

Contd.

Date	Days	Group III					Group IV					
		LB11	LB12	LB13	LB14	LB15	OR6	OR7	OR8	OR9	OR10	OR11
6/12/79	0	270	432	746	271	419	597	508	388	612	567	419
14/12/79	8	405	740	856	390	625	459	430	337	636	638	393
21/12/79	15	544	860	1136	489	600	638	361	376	473	625	388
28/12/79	22	486	566	793	393	553	558	424	397	531	719	424
3/1/80	28	664	678	992	551	807	549	602	467	669	722	467
11/1/80	36	1282	1627	1818	859	1627	965	788	719	897	965	924
18/1/80	43	834	1081	1411	724	1081	1089	728	641	832	895	743
25/1/80	50	884	1227	1391	568	788	1075	664	760	787	760	555
1/2/80	57	1764	868	1229	975	426	1068	627	627	707	814	667
8/2/80	64	1285	1655	2021	753	2077	1324	1136	892	1258	986	1272
15/2/80	71	1030	687	1245	587	856	2146	1669	1782	1831	1729	1900
22/2/80	78	1530	819	1818	759	946	1846	1075	1223	1206	1510	1019
26/2/80	82	1045	622	1480	466	861	1574	1072	966	1667	978	1139

Appendix A - Experiment 2

Individual plasma pepsinogen levels (m.u. of tyrosine) recorded at weekly intervals from four groups of lambs: Groups V, VI and VII were given two doses of 10,000 irradiated H. contortus L_3 and a dose of normal larvae with a four week interval between each dose. All four groups were challenged with 10,000 H. contortus L_3 four weeks after the last infection. Groups V and VI received anthelmintic treatment one week before challenge.

Date	Days	Group V					Group VI				
		Y58	Y82	Y83	Y84	Y85	P1	P2	P3	P41	P5
6/12/79	0	669	302	522	315	285	387	700	419	537	359
14/12/79	8	873	530	819	598	459	706	896	789	817	541
21/12/79	15	1070	459	764	487	486	558	1082	793	1441	614
28/12/79	22	952	380	659	393	326	620	967	580	913	393
3/1/80	28	1121	667	877	651	538	721	1095	721	1078	608
11/1/80	36	3350	1468	2306	1613	959	1817	2308	1498	2541	1380
18/1/80	43	1932	1053	1382	1150	820	930	1471	1221	1388	902
25/1/80	50	1679	1186	1419	1048	500	1083	1604	897	1138	680
1/2/80	57	841	948	1202	453	667	1114	1132	1360	1026	651
8/2/80	64	2872	2106	3459	1956	670	1694	2862	1457	2639	1287
15/2/80	71	1243	1700	2032	1474	616	1046	2191	1045	2161	1031
22/2/80	78	1246	1590	2462	1277	602	1189	1760	1264	1505	661
26/2/80	82	807	807	1058	675	373	702	966	1164	979	412
29/2/80	85	913	720	942	626	362	651	914	1211	707	486
7/3/80	92	2526	2567	5817	2248	942	1167	3114	2727	1740	941
13/3/80	98	1357	1074	2907	1195	697	1263	2409	2463	3109	858
20/3/80	105	1603	857	1279	942	674	1167	1814	3434	1829	815
25/3/80	110	2713	1442	1336	1018	514	1085	2199	1946	1628	727

Contd.

Date	Days	Group VII					Group VIII					
		R6	R7	R8	R9	R10	DB6	DB7	DB8	DB9	DB10	DB11
6/12/79	0	492	611	672	433	329	389	462	610	583	612	418
14/12/79	8	1185	706	910	613	522	327	486	582	547	560	352
21/12/79	15	891	682	970	861	585	386	443	612	624	610	471
28/12/79	22	1007	567	833	620	433	275	424	652	571	638	410
3/1/80	28	1162	565	1077	678	593	334	508	682	615	683	481
11/1/80	36	1541	2136	1905	1685	1541	435	586	793	774	692	600
18/1/80	43	1414	1066	1248	1233	1095	504	571	735	736	750	683
25/1/80	50	1149	887	1013	952	990	431	678	884	898	706	746
1/2/80	57	1119	1199	1636	1203	1205	359	694	694	627	667	694
8/2/80	64	NS	3203	1615	1916	2272	316	697	714	491	572	698
15/2/80	71	NS	1347	1647	2091	1963	464	827	887	626	627	928
22/2/80	78	NS	1987	1495	2235	1618	407	712	785	654	610	698
26/2/80	82	Died	2102	1107	1721	1475	320	676	689	464	570	755
29/2/80	85	-	1596	1156	1557	1540	341	769	662	515	608	701
7/3/80	92	-	4619	2992	4778	2846	777	1115	1465	993	872	2151
13/3/80	98	-	1183	1639	2314	2678	857	2307	2279	1448	1477	2349
20/3/80	105	-	1068	3152	2941	1617	554	1760	1030	844	1150	3788
25/3/80	110	-	1230	3033	2701	1110	656	1169	1041	843	1055	2564

Appendix A - Experiment 1

Individual faecal egg counts (e.p.g.) of four groups of lambs: Groups I, II and III were immunised with two doses of irradiated H. contortus L_3, Groups I and II received anthelmintic treatment three weeks after each vaccination. Four weeks after the second vaccination all four groups were given 10,000 normal L_3.

Date	Days After Infection	Group I					Group II				
		No. of Animal					No. of Animal				
		W6	W7	W8	W9	W10	LY26	LY27	LY28	LY29	LY36
6/12/79	0	0	0	0	0	0	0	0	0	0	0
21/12/79	15	0	0	0	0	0	0	0	0	0	0
24/12/79	18	0	0	0	0	0	0	0	0	0	0
3/1/80	28	0	0	0	0	0	0	0	0	0	0
8/1/80	33	0	0	0	0	0	0	0	0	0	0
15/1/80	40	0	0	0	0	0	0	0	0	0	0
22/1/80	47	0	0	0	0	0	0	0	0	0	0
29/1/80	54	0	0	0	0	0	0	0	0	0	0
5/2/80	61	0	0	0	0	0	0	0	0	0	0
12/2/80	68	0	0	0	0	0	0	0	0	0	0
19/2/80	75	5250	0	0	2250	0	0	150	50	0	0
28/2/80	84	21500	5500	29600	15600	500	350	14100	0	3800	0

Contd.

Date	Days After Infection	Group III					Group IV					
		No. of Animal					No. of Animal					
		LB11	LB12	LB13	LB14	LB15	OR6	OR7	OR8	OR9	OR10	OR11
6/12/79	0	0	0	0	0	0	0	0	0	0	0	0
21/12/79	15	0	0	0	0	0	0	0	0	0	0	0
24/12/79	18	0	0	0	0	0	0	0	0	0	0	0
3/1/80	28	0	0	0	0	0	0	0	0	0	0	0
8/1/80	33	0	0	0	0	0	0	0	0	0	0	0
15/1/80	40	0	0	0	0	0	0	0	0	0	0	0
22/1/80	47	0	0	0	0	0	0	0	0	0	0	0
29/1/80	54	0	0	0	0	0	0	0	0	0	0	0
5/2/80	61	0	0	0	0	0	0	0	0	0	0	0
12/2/80	68	0	0	0	0	0	0	0	0	0	0	0
19/2/80	75	0	0	0	150	4650	11000	50	31700	7250	650	2000
28/2/80	84	0	0	0	12000	34200	30000	5200	51000	39700	1400	12000

Appendix A Experiment 2 Individual faecal egg counts (e.p.g.) of four groups of lambs: Groups V, VI and VII were immunised with two doses of irradiated H. contortus L_3 and four weeks later immunised with 10,000 normal L_3. Three weeks later Groups V and VI received anthelmintic treatment and a week later all four groups were given 10,000 L_3.

Date	Days After Infection	Group V No. of Animal					Group VI No. of Animal				
		Y58	Y82	Y83	Y84	Y85	P1	P2	P3	P41	P5
6/12/79	0	0	0	0	0	0	0	0	0	0	0
21/12/79	15	0	0	0	0	0	0	0	0	0	0
24/12/79	18	0	0	0	0	0	0	0	0	0	0
3/1/80	28	0	0	0	0	0	0	0	0	0	0
8/1/80	33	0	0	0	0	0	0	0	0	0	0
15/1/80	40	0	0	0	0	0	0	0	0	0	0
22/1/80	47	0	0	0	0	0	0	0	0	0	0
29/1/80	54	0	0	0	0	0	0	0	0	0	0
5/2/80	61	0	0	0	0	0	0	0	0	0	0
12/2/80	68	0	0	0	0	0	0	0	0	0	0
19/2/80	75	0	0	0	0	0	0	0	0	0	0
28/2/80	84	0	0	0	0	0	0	0	0	0	0
4/3/80	89	0	0	0	0	0	0	0	0	0	0
11/3/80	96	0	0	0	0	0	0	0	0	0	0
17/3/80	102	0	0	0	0	0	0	0	0	0	0
25/3/80	110	0	0	0	0	2450	5950	50	10400	7200	18000
27/3/80	112	0	0	0	0	5400	10800	0	10000	15000	16000

Contd.

Date	Days After Infection	Group VII					Group VIII					
		No. of Animal					No. of Animal					
		R6	R7	R8	R9	R10	DB6	DB7	DB8	DB9	DB10	DB11
6/12/79	0	0	0	0	0	0	0	0	0	0	0	0
21/12/79	15	0	0	0	0	0	0	0	0	0	0	0
24/12/79	18	0	0	0	0	0	0	0	0	0	0	0
3/1/80	28	0	0	0	0	0	0	0	0	0	0	0
8/1/80	33	0	0	0	0	0	0	0	0	0	0	0
15/1/80	40	0	0	0	0	0	0	0	0	0	0	0
22/1/80	47	0	0	0	0	0	0	0	0	0	0	0
29/1/80	54	0	0	0	0	0	0	0	0	0	0	0
5/2/80	61	0	0	0	0	0	0	0	0	0	0	0
12/2/80	68	0	0	0	0	0	0	0	0	0	0	0
19/2/80	75	0	0	0	0	0	0	0	0	0	0	0
28/2/80	84	100	0	200	0	0	0	0	0	0	0	0
4/3/80	89	Died	1000	8200	400	50	0	0	0	0	0	0
11/3/80	96	-	400	3200	100	100	0	0	0	0	0	0
17/3/80	102	-	750	11000	300	850	0	150	0	1200	2900	4000
25/3/80	110	-	850	750	50	1800	12900	5600	100	46000	27300	8800
27/3/80	112	-	500	700	50	3300	30500	12600	100	31500	39800	6800

Appendix A - Experiment 3

Individual faecal egg counts (e.p.g.) of six groups of lambs: Groups I, II and III were given 10,000 40 kr irradiated, 60 kr irradiated and normal Cambridge strain H. contortus L_3 respectively. Groups IV, V and VI received 10,000 40 kr irradiated, 60 kr irradiated and normal Glasgow strain L_3 respectively.

Date	Days After Infection	Group I			Group II			Group III		
		No. of Animal			No. of Animal			No. of Animal		
		W11	W12	W13	P51	P52	P53	LB21	LB22	LB23
9/7/80	0	0	0	0	0	0	0	0	0	0
14/7/80	5	0	0	0	0	0	0	0	0	0
17/7/80	8	0	0	0	0	0	0	0	0	0
22/7/80	13	0	0	0	0	0	0	0	0	0
24/7/80	15	0	0	0	0	0	0	0	0	0
26/7/80	17	0	0	0	0	0	0	0	0	0
28/7/80	19	0	0	0	0	0	0	100	2600	10000
30/7/80	21	0	0	0	0	0	0	1350	2150	9800
1/8/80	23	0	0	0	0	0	0	4200	5000	14000
4/8/80	26	0	0	0	0	0	0	8600	11300	17000

Contd.

Date	Days After Infection	Group IV			Group V			Group VI		
		No. of Animal			No. of Animal			No. of Animal		
		R34	R35	R36	LY81	LY82	LY83	FR73	FR74	FR75
9/7/80	0	0	0	0	0	0	0	0	0	0
14/7/80	5	0	0	0	0	0	0	0	0	0
17/7/80	8	0	0	0	0	0	0	0	0	0
22/7/80	13	0	0	0	0	0	0	0	0	0
24/7/80	15	0	0	0	0	0	0	0	0	0
26/7/80	17	0	0	0	0	0	0	1300	1300	1500
28/7/80	19	0	0	0	0	0	0	16300	8800	15900
30/7/80	21	0	0	0	0	0	0	41500	17500	29500
1/8/80	23	0	0	0	0	0	0	36000	15700	38900
4/8/80	26	0	0	0	0	0	0	48400	24600	65200

Appendix A - Experiment 4

Individual faecal egg counts (e.p.g.) of four groups of lambs: Groups VII and IX were immunised with two doses of γ-irradiated Glasgow and Cambridge strain *H. contortus* L_3 respectively. Four weeks after the second vaccination Groups VII and VIII were given 10,000 normal Glasgow strain L_3 and Groups IX and X received 10,000 normal Cambridge strain L_3.

Date	Days After Infection	Group VII No. of Animal				Group VIII No. of Animal			
		R67	R68	R69	R70	DB69	DB70	DB71	DB72
23/9/80	0	0	0	0	0	0	0	0	0
30/9/80	7	0	0	0	0	0	0	0	0
7/10/80	14	0	0	0	0	0	0	0	0
14/10/80	21	0	0	0	0	0	0	0	0
20/10/80	27	0	0	0	0	0	0	0	0
27/10/80	34	0	0	0	0	0	0	0	0
3/11/80	41	0	0	0	0	0	0	0	0
10/11/80	48	0	0	0	0	0	0	0	0
17/11/80	55	0	0	0	0	0	0	0	0
24/11/80	62	0	0	0	0	0	0	0	0
1/12/80	69	0	0	0	0	0	0	0	0
8/12/80	76	0	0	0	9200	20600	3250	0	11100
11/12/80	79	0	400	0	17800	29300	10900	100	24800
16/12/80	84	9900	50	900	18300	57700	18100	200	38500

Contd.

Date	Days After Infection	Group IX				Group X			
		No. of Animal				No. of Animal			
		P77	P78	P79	P80	LY73	LY74	LY75	LY76
23/9/80	0	0	0	0	0	0	0	0	0
30/9/80	7	0	0	0	0	0	0	0	0
7/10/80	14	0	0	0	0	0	0	0	0
14/10/80	21	0	0	0	0	0	0	0	0
20/10/80	27	0	0	0	0	0	0	0	0
27/10/80	34	0	0	0	0	0	0	0	0
3/11/80	41	0	0	0	0	0	0	0	0
10/11/80	48	0	0	0	0	0	0	0	0
17/11/80	55	0	0	0	0	0	0	0	0
24/11/80	62	0	0	0	0	0	0	0	0
1/12/80	69	0	0	0	0	0	0	0	0
8/12/80	76	750	150	1950	50	700	2850	0	0
11/12/80	79	1850	850	5100	750	2400	4150	3050	350
16/12/80	84	3100	800	6500	1100	1550	4100	4350	600

Appendix A - Experiment 5

Individual faecal egg counts (e.p.g.) of four groups of lambs: Groups XI and XIII were immunised with two doses of γ - irradiated H. contortus L_3 of Glasgow and Cambridge strain respectively. Four weeks after the second vaccination Groups XI and XII received 10,000 normal Glasgow strain L_3 while Groups XIII and XIV were given 10,000 normal Cambridge strain L_3.

Date	Days After Infection	Group XI No. of Animal				Group XII No. of Animal			
		W4	W8	Y11	B24	W5	W7	W10	Y19
23/9/80	0	0	0	0	0	0	0	0	0
1/10/80	8	0	0	0	0	0	0	0	0
8/10/80	15	0	0	0	0	0	0	0	0
15/10/80	22	0	0	0	0	0	0	0	0
22/10/80	29	0	0	0	0	0	0	0	0
29/10/80	36	0	0	0	0	0	0	0	0
5/11/80	43	0	0	0	0	0	0	0	0
12/11/80	50	0	0	0	0	0	0	0	0
19/11/80	57	0	0	0	0	0	0	0	0
26/11/80	64	0	0	0	0	0	0	0	0
3/12/80	71	0	0	0	0	0	0	0	0
8/12/80	76	4	338	263	163	950	6875	3425	6200
10/12/80	78	0	1688	600	1413	1213	10200	2875	9000
12/12/80	80	0	4216	1365	3105	1875	7812	3100	11780
15/12/80	83	0	6076	2760	1800	1350	8060	3210	5084
16/12/80	84	0	3100	2460	1980	885	2356	3270	5704

Contd.

Date	Days After Infection	Group XIII				Group XIV			
		No. of Animal				No. of Animal			
		W9	Y14	Y18	B22	Y13	B21	B23	B25
23/9/80	0	0	0	0	0	0	0	0	0
1/10/80	8	0	0	0	0	0	0	0	0
8/10/80	15	0	0	0	0	0	0	0	0
15/10/80	22	0	0	0	0	0	0	0	0
22/10/80	29	0	0	0	0	0	0	0	0
29/10/80	36	0	0	0	0	0	0	0	0
5/11/80	43	0	0	0	0	0	0	0	0
12/11/80	50	0	0	0	0	0	0	0	0
19/11/80	57	0	0	0	0	0	0	0	0
26/11/80	64	0	0	0	0	0	0	0	0
3/12/80	71	0	0	0	0	0	0	0	0
8/12/80	76	0	138	475	613	763	1413	1688	1138
10/12/80	78	125	913	950	1050	1388	1888	2650	1238
12/12/80	80	NS	645	975	1485	1215	2190	2715	2865
15/12/80	83	510	1155	3270	1695	2220	1185	2728	1920
16/12/80	84	150	810	555	1365	3596	2070	2160	2415

N.S. No sample

Appendix A - Experiment 6

Individual faecal egg counts (e.p.g.) of two groups of lambs: Group I received two immunising infections of normal Glasgow strain *H. contortus* L_3. Three weeks after the second infection Groups I and II were given anthelmintic treatment and one week later both groups received 10,000 normal L_3.

Date	Days After Infection	Group I			Group II		
		No. of Animal			No. of Animal		
		R41	R42	R43	G47	G56	G57
16/2/81	-1	0	0	0	0	0	0
17/2/81	0	0	0	0	0	0	0
24/2/81	7	0	0	0	0	0	0
3/3/81	14	0	0	0	0	0	0
10/3/81	21	7700	850	1950	0	0	0
17/3/81	28	18200	4100	22700	0	0	0
24/3/81	35	6800	1600	5900	0	0	0
31/3/81	42	11500	250	2700	0	0	0
7/4/81	49	8400	100	1250	0	0	0
14/4/81	56	0	0	0	0	0	0
21/4/81	63	0	0	0	0	0	0
28/4/81	70	0	0	0	0	0	0
5/5/81	77	5400	50	0	4600	14700	25900
12/5/81	84	17900	150	50	24300	26500	71900

Appendix A - Experiment 7

Individual faecal egg counts (e.p.g.) of six groups of lambs: Groups III and IV were immunised with two doses of Glasgow strain H. contortus L_3 irradiated at 40 kr with γ - rays and X - rays respectively. Groups VII and VIII were immunised with two doses of MSD strain H. contortus L_3 irradiated at 40 kr with γ - rays and X - rays respectively. Four weeks after the second vaccination Groups III, IV and V were given 10,000 normal Glasgow strain L_3 while Groups VI, VII and VIII were given 10,000 normal MSD L_3.

Date	Days After Infection	Group III				Group IV				Group V			
		No. of Animal				No. of Animal				No. of Animal			
		DY77	DY78	DY79	DY80	P56	P57	P58	P59	LY58	LY59	LY60	LY65
3/3/81	0	0	0	0	0	0	0	0	0	0	0	0	0
10/3/81	7	0	0	0	0	0	0	0	0	0	0	0	0
17/3/81	14	0	0	0	0	0	0	0	0	0	0	0	0
24/3/81	21	0	0	0	0	0	0	0	0	0	0	0	0
31/3/81	28	0	0	0	0	0	0	0	0	0	0	0	0
7/4/81	35	0	0	0	0	0	0	0	0	0	0	0	0
14/4/81	42	0	0	0	0	0	0	0	0	0	0	0	0
21/4/81	49	0	0	0	0	0	0	0	0	0	0	0	0
28/4/81	56	0	0	0	0	0	0	0	0	0	0	0	0
5/5/81	63	0	0	0	0	0	0	0	0	0	0	0	0
12/5/81	70	0	0	0	0	0	0	0	0	0	0	0	0
19/5/81	77	0	0	0	0	0	0	0	0	15900	6250	7900	50
26/5/81	84	0	0	0	0	0	0	0	0	20700	9750	Died	5300

Date	Days After Infection	Group VI				Group VII				Group VIII			
		No. of Animal				No. of Animal				No. of Animal			
		OR11	OR13	OR14	OR15	DB12	DB14	DB21	DB22	R37	R38	R39	R40
3/3/81	0	0	0	0	0	0	0	0	0	0	0	0	0
10/3/81	7	0	0	0	0	0	0	0	0	0	0	0	0
17/3/81	14	0	0	0	0	0	0	0	0	0	0	0	0
24/3/81	21	0	0	0	0	0	0	0	0	0	0	0	0
31/3/81	28	0	0	0	0	0	0	0	0	0	0	0	0
7/4/81	35	0	0	0	0	0	0	0	0	0	0	0	0
14/4/81	42	0	0	0	0	0	0	0	0	0	0	0	0
21/4/81	49	0	0	0	0	0	0	0	0	0	0	0	0
28/4/81	56	0	0	0	0	0	0	0	0	0	0	0	0
5/5/81	63	0	0	0	0	0	0	0	0	0	0	0	0
12/5/81	70	0	0	0	0	0	0	0	0	0	0	0	0
19/5/81	77	0	0	0	0	0	0	200	0	700	0	45600	11800
26/5/81	84	50	1475	0	0	5175	4325	3400	50	32825	8550	39450	9275

Appendix A - Experiment 8

Individual faecal egg counts (e.p.g.) of five groups of lambs: Groups IX and X were immunised with two doses of Glasgow strain H.contortus L_3 γ - irradiated at 40 kr and 60 kr respectively. Group XII was immunised with two doses of 40 kr γ - irradiated MSD strain L_3. Four weeks after the second vaccination Groups IX, X and XI received 10,000 normal Glasgow strain L_3 while Groups XII and XIII received 10,000 normal MSD L_3.

Date	Days After Infection	Group IX					Group X					Group XI				
		No. of Animal					No. of Animal					No. of Animal				
		R1	R2	R3	R4	R5	P11	P12	P13	P15	P16	FR11	FR12	FR13	FR14	FR15
9/12/81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16/12/81	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22/12/81	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30/12/81	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6/1/82	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13/1/82	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20/1/82	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27/1/82	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3/2/82	56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10/2/82	63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19/2/82	72	0	0	0	0	0	0	0	0	0	0	1200	1250	0	300	450
22/2/82	75	0	0	0	0	0	0	0	0	0	0	12000	14000	750	2900	7000
24/2/82	77	0	0	0	0	0	0	0	0	0	0	15900	12800	5700	4900	6900
26/2/82	79	0	0	0	150	0	50	0	0	0	350	32300	9800	6900	8900	10100
1/3/82	82	0	0	0	400	0	0	400	0	0	700	26800	4250	7000	6100	9700
3/3/82	84	0	0	0	5010	0	50	900	0	0	850	35600	8700	7200	9600	12200

Contd.

Date	Days After Infection	Group XII					Group XIII				
		No. of Animal					No. of Animal				
		G11	G12	G13	G14	G15	DY1	DY2	DY3	DY4	DY5
9/12/81	0	0	0	0	0	0	0	0	0	0	0
16/12/81	7	0	0	0	0	0	0	0	0	0	0
22/12/81	13	0	0	0	0	0	0	0	0	0	0
30/12/81	21	0	0	0	0	0	0	0	0	0	0
6/1/82	28	0	0	0	0	Died	0	0	0	0	0
13/1/82	35	0	0	Died	0	-	0	0	0	0	0
20/1/82	42	0	0	-	0	-	0	0	0	0	0
27/1/82	49	0	0	-	0	-	0	0	0	0	0
3/2/82	56	0	0	-	0	-	0	0	0	0	0
10/2/82	63	0	0	-	0	-	0	0	0	0	0
19/2/82	72	600	150	-	NS	-	600	200	250	0	750
22/2/82	75	NS	50	-	2850	-	11800	8500	3400	2400	10900
24/2/82	77	6400	50	-	5200	-	14900	17600	6600	5600	15000
26/2/82	79	8800	50	-	8600	-	18100	20800	7300	11500	16300
1/3/82	82	27300	50	-	27100	-	42200	29200	12100	15600	36200
3/3/82	84	22500	50	-	13500	-	33800	31200	11500	14200	31500

N.S. No sample

Appendix A - Experiment 1

Individual worm burdens of four groups of lambs: Groups I, II and III were immunised with two doses of γ - irradiated *H. contortus* L_3 and challenged with 10,000 L_3. Groups I and II received anthelmintic treatment three weeks after each vaccination. Group IV received only the challenge infection.

Group	Animal No.	Immature stages L_4/L_5	Stunted adult females	Adults females	Adults males	Total worm burden
I	W 6	0	0	1700	1200	2900
	7	0	0	2200	2800	5000
	8	0	0	1700	600	2300
	9	0	0	2600	2100	4700
	10	0	0	350	400	750
II	LY 26	400	0	700	900	2000
	27	0	0	800	500	1300
	28	0	0	750	350	1100
	29	0	0	2200	1600	3800
	36	0	0	0	0	0
III	LB 11	800	0	0	0	800
	12	0	650	0	350	1000
	13	0	2000	400	1950	4350
	14	0	350	250	50	650
	15	0	2300	1400	1900	5600
IV	OR 6	0	0	2550	2200	4750
	7	0	0	1250	500	1750
	8	0	0	1450	1750	3200
	9	0	0	2100	1800	3900
	10	0	0	750	150	900
	11	0	0	1150	750	1900

Appendix A - Experiment 2

Individual worm burdens of four groups of lambs: Group V, VI and VII were given two doses of 10,000 γ - irradiated *H. contortus* L_3 followed by normal L_3 with a four week interval between each dose. Four weeks after the last infection all four groups were given 10,000 L_3. Group V and VI received anthelmintic treatment one week before the last infection.

Group	Animal No.	Immature stages L_4/L_5	Stunted adult females	Adults females	Adults males	Total worm burden
V	Y 58	0	0	950	600	1550
	82	0	0	200	200	400
	83	1750	0	150	200	2100
	84	400	0	150	200	750
	85	50	0	1600	1200	2850
VI	P 1	0	0	2500	2000	4500
	2	1400	0	0	50	1450
	3	0	0	600	1100	1700
	41	0	0	1800	500	2300
	5	0	0	1200	1200	2400
VII	R 6*	0	0	350	200	550
	7	0	0	100	50	150
	8	450	0	250	150	850
	9	650	0	150	50	850
	10	1050	0	100	350	1500
VIII	DB 6	0	0	1000	1300	2300
	7	0	0	900	350	1250
	8	0	0	0	0	0
	9	0	0	1300	2000	3300
	10	0	0	2200	2050	4250
	11	0	0	600	600	1200

* Died before the last challenge infection.

Appendix A - Experiment 3

Individual worm burdens of six groups of lambs: Groups I, II and III received 10,000, 40 kr γ - irradiated, 60 kr γ - irradiated and normal Cambridge strain *H. contortus* L_3 respectively. Groups IV, V and VI received 10,000, 40 kr γ - irradiated, 60 kr γ - irradiated and normal Glasgow strain *H. contortus* L_3 respectively.

Group	Animal No.		Immature stages L_4/L_5	Stunted adult females	Adults females	Adults males	Total worm burdens
I	W	11	0	1000	0	0	1000
		12	0	350	0	0	350
		13	0	1350	0	0	1350
II	P	51	0	1000	0	0	1000
		52	0	1300	0	0	1300
		53	0	500	0	0	500
III	LB	21	0	0	850	400	1250
		22	0	0	750	300	1050
		25	0	0	1300	1000	2300
IV	R	34	0	600	0	0	600
		35	0	500	0	0	500
		36	0	1100+100*	0	0	1200
V	LY	81	0	550	0	0	550
		82	0	1050	0	0	1050
		83	0	350	0	0	350
VI	FR	73	0	0	1150	900	2050
		74	0	0	1100	800	1900
		75	0	0	1600	1650	3250

* No. of stunted males recovered.

Appendix A - Experiment 4

Individual worm burdens of four groups of lambs: Groups VII and IX were immunised with two doses of 60 kr γ - irradiated Glasgow and Cambridge strain H. contortus larvae respectively. Four weeks after the second vaccination Groups VII and VIII received 10,000 normal Glasgow strain L_3 and Groups IX and X received 10,000 normal Cambridge strain L_3.

Group	Animal No.		Immature stages L_4/L_5	Stunted adult females	Adults females	Adults males	Total worm burden
VII	R	67	0	0	1950	2050	4000
		68	0	0	0	0	0
		69	0	0	200	50	250
		70	0	0	1300	1150	2450
VIII	DB	69	0	0	2850	2600	5450
		70	0	0	1000	950	1950
		71	0	0	50	50	100
		72	0	0	1700	1800	3500
IX	P	77	150	0	500	350	1000
		78	150	0	100	100	350
		79	150	0	400	350	900
		80	150	0	150	150	450
X	LY	73	750	0	200	150	1100
		74	400	0	700	300	1400
		75	150	0	950	1150	2250
		76	200	0	50	50	300

Appendix A - Experiment 5

Individual worm burdens of four groups of lambs: Groups XI and XIII were immunised with two doses of 60 kr γ - irradiated Glasgow and Cambridge strain H. contortus larvae respectively. Four weeks after the second vaccination Groups XI and XII received 10,000 normal Glasgow strain L_3 and groups XIII and XIV received 10,000 normal Cambridge strain L_3.

Group	Animal No.		Immature stages L_4/L_5	Adults females	Adults males	Total worm burden
XI	W	4	50	1425	1388	2863
	W	8	0	3877	3386	7263
	Y	11	290	4370	3937	8597
	B	24	122	3855	2902	6879
XII	W	5	0	579	386	965
	W	7	0	4177	4164	8341
	W	10	177	1923	1924	4024
	Y	19	135	5480	5069	10684
XIII	W	9	51	167	136	354
	Y	14	243	445	342	1030
	Y	18	498	714	592	1816
	B	12	176	425	225	826
XIV	Y	13	14	590	458	1062
	B	21	14	1042	810	1866
	B	23	15	476	381	872
	B	25	39	928	1048	2015

Appendix A - Experiment 6

Individual worm burdens of two groups of lambs: Group I was immunised with two doses of normal Glasgow strain *H. contortus* L_3. Three weeks after the second infection Groups I and II received anthelmintic treatment and one week later both groups were given 10,000 normal L_3.

Group	Animal No.	Larval stages L_4/L_5	Adults females	Adults males	Total worm burden
I	R 41	0	2700	2250	4950
	42	50	150	0	200
	43	50	500	250	800
II	G 47	0	1350	450	1800
	56	0	2650	2950	5600
	57	0	2650	2600	5250

Appendix A - Experiment 7

Individual worm burdens of six groups of lambs: Groups III and IV were immunised with two doses of Glasgow strain *H. contortus* L_3 irradiated at 40 kr with γ - rays and X - rays respectively. Groups VI and VII were immunised with two doses of MSD strain *H. contortus* L_3 irradiated at 40 kr with γ - rays and X - rays respectively. Four weeks after the second vaccination Groups III, IV and V were given 10,000 normal Glasgow strain L_3 while Groups VI, VII and VIII were given 10,000 normal MSD L_3.

Group	Animal No.	Immature stages L_4/L_5	Stunted adult females	Adults females	Adults males	Total worm burden
III	DY 77	0	100	0	0	100
	78	0	50	0	0	50
	79	0	100	0	0	100
	80	0	50	0	0	50
IV	P 56	0	300	0	300	600
	57	0	50	0	0	50
	58	0	250	0	100	350
	59	0	50	0	0	50
V	LY 58	0	0	3050	3550	6600
	59	0	0	2450	1700	4150
	60*	-	-	-	-	-
	65	0	0	1950	1800	3750
VI	OR 12	0	950	200	550	1700
	13	0	2300	500	2400	5200
	14	0	200	0	250	450
	15	0	0	0	0	0
VII	DB 12	0	1050	400	750	2200
	14	0	2350	650	1500	4500
	21	0	2600	1050	2250	5900
	22	0	300	50	50	400
VIII	R 37	0	0	2300	1950	4250
	38	0	0	2650	2200	4850
	39	0	0	2100	2150	4250
	40	0	0	1700	1250	2950

*Died before the end of the experiment

Appendix A - Experiment 8

Individual worm burdens of five groups of lambs: Group IX and X were immunised with two doses of Glasgow strain *H. contortus* L_3 γ - irradiated at 40 kr and 60 kr respectively. Group XII was immunised with two doses of MSD strain *H. contortus* L_3 γ - irradiated at 40 kr. Four weeks after the second vaccination Groups IX, X and XI received 10,000 normal Glasgow strain *H. contortus* L_3 while groups XII and XIII received 10,000 normal MSD L_3.

Group	Animal No.		Immature stages L_4/L_5	Stunted adult females	Adults females	Adults males	Total worm burden
IX	R	1	0	50	0	0	50
		2	0	0	0	0	0
		3	0	50	0	0	50
		4	0	200	150	200	550
		5	0	250	0	100	350
X	P	11	0	50	0	50	100
		12	50	100	200	650	1000
		13	0	100	150	0	250
		15	0	0	0	0	0
		16	150	500	400	650	1700
XI	FR	11	0	0	1400	1800	3200
		12	0	0	1400	750	2150
		13	0	0	1700	1450	3150
		14	0	0	1000	1500	2500
		15	0	0	850	900	1750
XII	G	11	0	400	700	550	1650
		12	0	50	50	50	150
		13*	-	-	-	-	-
		14	0	650	400	900	1950
		15*	-	-	-	-	-
XIII	DY	1	0	0	950	800	1750
		2	0	0	850	850	1700
		3	0	0	1000	1150	2150
		4	0	0	1200	850	2050
		5	0	0	1100	1300	2400

* Died before the termination of the experiment

APPENDIX B

Appendix B

Nippostrongylus brasiliensis infection in the rat

Nippostrongylus brasiliensis is a trichostrongyle nematode found in the small intestine of wild rats (Yokogawa, 1920; Haley, 1961) from which it has been isolated in many parts of the world. Yokogawa (1920) called this parasite *Heligmosomum muris*. Later the name was changed to *Nippostrongylus muris* and finally to *Nippostrongylus brasiliensis* (Haley, 1961).

N. brasiliensis infection in rats is widely used as a model system for studying many aspects of helminthology. The main reason for its widespread use is because the life cycle and the host response resemble those of similar nematodes which are of medical and veterinary importance (Ogilvie and Jones, 1971). Additional advantages of using this host/parasite system are the short life cycle and the ready availability and cheapness of the laboratory rat.

The life cycle and the development of *N. brasiliensis* was first described by Yokogawa (1922) and later Haley (1961, 1962) gave a detailed description of the systematics, hosts, geographic distribution and preparasitic development of this parasite together with a description of the parasitic stages in the laboratory rat.

A brief description of the life cycle is as follows: adult worms live in the small intestine and mature female parasites produce eggs which are embryonated when passed with the faeces of the infected host. These eggs hatch and develop through two larval stages (L_1 and L_2) and two moults before reaching the infective third stage (L_3) in three to four days at room temperature. The L_3 is not ensheathed i.e. it does not retain the cuticle of the previous stage.

After natural percutaneous or experimental subcutaneous infection of the rat the larvae migrate to the lungs where they are found between 11-15 hours after infection. The exact route of migration is not

yet known, but is believed to be via the lymph and blood (Ogilvie and Jones, 1971). During the migration to the lungs a proportion of the larvae are lost (Jarrett et al, 1968). Larvae which reach the lungs break into the alveoli and migrate into the air passages where they grow and undergo the third moult between 32-46 hours after infection to become fourth stage larvae (L_4). The L_4 migrate up the trachea and are swallowed and enter the small intestine 45-60 hours post-infection. There they undergo rapid growth followed by the final moult which occurs between 90 and 123 hours after infection. After fertilisation by the male, egg production by the mature female worm begins by the end of fifth day after infection.

In a primary infection over 90% of the larvae which migrate from the lungs to the intestine reach maturity (Love, Kelly and Dineen, 1974). The adult worms live in the anterior small intestine, predominantly in the proximal jejunum (Murray, 1972) where they are found coiled around the villi. The precise mode of feeding of the parasite remains obscure. Mulligan et al (1965) and Neilson (1969) have shown conclusively that N. brasiliensis adult worms neither suck blood nor do their feeding activities cause much blood loss from intestinal damage. It has been suggested that the parasite feeds on intestinal debris and contents. This is thought to be accomplished by the nematode bracing itself against the villi while parasite enzymes from the buccal cavity are poured on to the host cells. The resultant cellular debris and contents is then ingested by the pumping action of the oesophagus (Lee, 1969, 1970).

After reaching the intestine the adult worm population is static for about one week. The faecal egg count rises rapidly from day 6 to day 10 after infection: thereafter the egg count starts to fall reaching zero by 15-20 days. In parallel with this the worm population found in the intestine decreases gradually between days 10

and 12 and rapidly therafter, until few worms remain by day 20 (Jarrett et al, 1968; Murray et al, 1971).

This phenomenon of loss of the primary infection is often termed 'self-cure' and was first described in N. brasiliensis infection by Africa (1931). Later Taliaferro and Sarles (1937) demonstrated that the loss of the worm burdens was due to an immune reaction by the host and not to ageing of the worms. The precise timing of the various stages in the life cycle and of the expulsion can vary to some extent depending on the breed, strain and sex of the host rat. Also with very small infections of about 50 larvae, worm loss is gradual and extended over a 30 day period (Haley and Parker, 1961; Jarrett et al, 1968).

After a primary infection the host develops resistance to subsequent infection with N. brasiliensis. The magnitude of the resistance which develops to reinfection is variable and depends on several factors. These include: the age of the host at the time of infection, the interval between primary and subsequent infections and the number of reinfections given (Ogilvie and Jones, 1971). Resistance to reinfection with N. brasiliensis is marked by a decrease in egg output and duration of egg production after challenge, stunting of the worms which do develop in the intestine and their accelerated expulsion within two to three days after reaching the small intestine (Jarrett and Urquhart, 1971).

Appendix B Table 1

Experiment 1: First experimental attempt to produce a 'moderate' degree of immunity to N. brasiliensis with low sensitising infections.

Groups A, B and C were immunised with 50 L_3, 100 L_3 and 500 L_3 respectively and 24 days later all four groups received 3000 L_3.

Individual worm burdens on day seven after challenge infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
A	1	6	62	56	124
	2	11	28	15	54
	3	2	8	5	15
	4	2	10	4	16
	5	3	28	10	41
B	1	12	312	278	602
	2	17	36	23	76
	3	9	16	8	33
	4	13	31	10	54
C	1	9	73	90	172
	2	32	72	26	130
	3	15	28	12	55
	4	13	18	10	41
D	1	1	486	320	807
	2	3	1326	810	2139
	3	1	939	533	1473
	4	9	1435	1041	2485
	5	9	534	282	825

Contd.

Individual worm burdens on day 10 after challenge.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
A	1	0	2	1	3
	2	0	0	0	0
	3	1	1	0	2
	4	0	1	0	1
	5	0	2	0	2
B	1	0	0	0	0
	2	2	10	5	17
	3	2	2	1	5
	4	0	1	0	1
	5	0	1	0	1
C	1	0	0	0	0
	2	0	0	0	0
	3	0	1	1	2
	4	0	0	1	1
	5	0	0	1	1
D	1	0	1088	690	1778
	2	0	1330	910	2240
	3	0	937	603	1540
	4	0	1083	857	1940
	5	0	1136	784	1920

Contd.

Individual worm burdens on day 14 after challenge.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
A	1	0	0	0	0
	2	0	22	36	58
	3	0	0	0	0
	4	3	4	13	20
	5	0	1	2	3
B	1	0	1	4	5
	2	0	0	2	2
	3	0	1	1	2
	4	0	0	0	0
C	1	0	4	10	14
	2	2	6	11	19
	3	0	1	3	4
	4	0	2	1	3
D	1	0	100	240	340
	2	0	161	156	317
	3	0	83	114	197
	4	0	87	121	208
	5	0	103	175	278

Appendix B Table 2

Experiment 2: Second experimental attempt to produce a 'moderate' degree of immunity to N. brasiliensis with low sensitising infections.

Groups E, F, G and H were immunised with 100 L_3, 50 L_3, 25 L_3 and 10 L_3 respectively and 24 days later all five groups received 3000 L_3.

Individual worm burdens 11 days after primary infection.

Group	Rat No.	Adults			Total
		Females	Males		
E	1	21	18		39
	2	35	25		60
				Mean	50
F	1	14	17		31
	2	14	16		30
				Mean	31
G	1	6	7		13
	2	8	8		16
				Mean	15
H	1	1	3		4
	2	4	2		6
				Mean	5

Individual worm burdens at the time of challenge

Group	Rat No.	Adults			Total
		Females	Males		
E	1	8	21		29
	2	3	16		19
				Mean	24
F	1	10	9		19
	2	9	11		20
				Mean	20
G	1	4	6		10
	2	3	6		9
				Mean	10
H	1	2	1		3
	2	1	2		3
				Mean	3

Contd.

Individual worm burdens four days after challenge infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
E	1	78	0	12	90
	2	235	5	17	257
	3	448	4	9	461
	4	63	0	2	65
	5	14	0	0	14
F	1	77	0	2	79
	2	208	0	2	210
	3	576	6	10	592
	4	655	1	1	657
	5	926	5	10	941
G	1	166	0	0	166
	2	552	2	4	558
	3	60	2	4	66
	4	874	0	0	874
	5	620	0	2	622
H	1	1778	0	2	1780
	2	1808	2	0	1810
	3	1772	0	0	1772
	4	1788	0	2	1790
	5	1776	0	0	1776
I	1	2008	0	0	2008
	2	1790	0	0	1790
	3	2080	0	0	2080
	4	2044	0	0	2044
	5	1786	0	0	1786

Contd.

Individual worm burdens seven days after challenge infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
E	1	6	188	50	244
	2	0	6	8	14
	3	0	6	4	10
	4	0	14	6	20
	5	8	12	8	28
F	1	10	36	8	54
	2	2	14	10	26
	3	4	44	4	52
	4	12	76	16	104
	5	6	30	4	40
G	1	36	146	28	210
	2	40	84	14	138
	3	16	116	28	160
	4	12	60	22	94
	5	8	88	24	120
H	1	24	60	4	88
	2	0	54	6	60
	3	12	174	50	236
	4	8	406	52	466
	5	0	460	56	516
I	1	0	632	362	994
	2	0	948	910	1858
	3	0	1030	618	1648
	4	2	416	380	798
	5	0	1008	636	1644

Contd.

Individual worm burdens 10 days after challenge infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
E	1	0	4	14	18
	2	2	10	38	50
	3	0	4	4	8
	4	2	6	20	28
	5	6	2	8	16
	6	2	0	16	18
F	1	0	0	0	0
	2	2	2	36	40
	3	0	2	26	28
	4	2	0	16	18
	5	0	2	8	10
	6	0	0	4	4
G	1	0	4	6	10
	2	0	2	8	10
	3	0	22	70	92
	4	0	2	2	4
	5	0	0	4	4
	6	4	2	34	40
H	1	0	0	6	6
	2	12	0	16	28
	3	0	0	0	0
	4	0	0	34	34
	5	0	0	0	0
	6	0	0	16	16
I	1	0	864	468	1332
	2	0	756	334	1090
	3	0	788	364	1152
	4	0	700	436	1136
	5	0	760	418	1178
	6	0	860	416	1276

Appendix B Table 3

Experiment 3: The effect of anthelmintic treatment on the response to reinfection one week later.

Groups J, K a, L a and M a received a primary infection of 10 L_3 and four weeks later all eight groups were given 3000 L_3. One week before the challenge Groups K a, b, L a, b and M a, b received anthelmintic treatment.

Individual worm burdens four days after the challenge infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
J	1	914	1	1	916
	2	552	0	2	554
	3	1440	2	1	1443
	4	738	1	1	740
	5	1096	2	0	1098
K a	1	1430	-	-	1430
	2	1734	-	-	1734
	3	708	-	-	708
	4	1190	-	-	1190
	5	1574	-	-	1574
L a	1	1290	-	-	1290
	2	1296	-	-	1296
	3	1570	-	-	1570
	4	1840	-	-	1840
	5	1526	-	-	1526
M a	1	1900	-	-	1900
	2	1687	-	-	1687
	3	1951	-	-	1951
	4	1330	-	-	1330
	5	1790	-	-	1790
N	1	2004	-	-	2004
	2	2040	-	-	2040
	3	2112	-	-	2112
	4	1908	-	-	1908
	5	1030	-	-	1030
K b	1	1320	-	-	1320
	2	1070	-	-	1070
L b	1	2220	-	-	2220
	2	1900	-	-	1900
M b	1	1824	-	-	1824
	2	1892	-	-	1892

Contd.

Individual worm burdens seven days after the challenge infection.

Group	Rat No.	Larval stages	Adults Females	Adults Males	Total
J	1	48	160	44	252
	2	76	64	28	148
	3	90	226	78	394
	4	104	108	40	252
	5	140	24	10	174
K a	1	12	10	4	26
	2	140	164	42	346
	3	20	10	2	32
	4	200	122	22	344
	5	2	4	4	10
L a	1	256	424	146	826
	2	50	560	184	794
	3	66	22	8	96
	4	170	56	32	258
	5	148	180	40	368
M a	1	120	128	24	272
	2	58	116	38	212
	3	24	40	10	74
	4	102	312	94	508
	5	124	258	92	474
N	1	2	1092	540	1634
	2	4	1002	468	1474
	3	6	1320	728	2054
	4	4	1056	486	1546
	5	4	1248	612	1864
K b	1	4	956	456	1416
	2	6	1068	480	1554
	3	6	752	374	1132
L b	1	14	934	552	1500
	2	2	968	678	1648
	3	8	968	610	1586
M b	1	16	1238	896	2150
	2	20	1176	534	1730
	3	14	1160	570	1744

Contd.

Individual worm burdens 10 days after challenge infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
J	1	8	44	58	110
	2	18	24	40	82
	3	2	10	30	42
	4	8	34	56	98
	5	4	8	6	18
K a	1	0	10	28	38
	2	0	2	4	6
	3	0	2	6	8
	4	0	0	2	2
	5	0	0	2	2
L a	1	2	2	8	12
	2	0	0	4	4
	3	2	0	6	8
	4	0	0	4	4
	5	0	12	58	70
M a	1	0	2	6	8
	2	0	0	6	6
	3	2	2	8	12
	4	2	6	16	24
	5	2	2	8	12
N	1	2	300	560	862
	2	8	448	940	1396
	3	10	610	1032	1652
	4	6	480	906	1392
	5	6	330	588	924
K b	1	0	134	260	394
	2	4	312	642	958
L b	1	0	432	800	1232
	2	0	424	818	1242
M b	1	0	470	1220	1690
	2	0	452	974	1426

Contd.

Individual worm burdens of rats from Group J on day 17 after infection.

Group	Rat No.	Larval stages	Adults Females	Adults Males		Total
J	1	-	1	2		3
	2	-	3	4		7
	3	-	3	3		6
	4	-	1	2		3
					Mean	5

Individual worm burdens of rats from Groups J, K a, L a and M a at the time of challenge.

Group	Rat No.	Larval stages	Adults Females	Adults Males		Total
J	1	-	2	2		4
	2	-	1	3		4
	3	-	1	5		6
	4	-	1	3		4
					Mean	5
K a	1	-	-	-		-
	2	-	-	-		-
L a	1	-	-	1		1
	2	-	-	-		-
					Mean	1
M a	1	-	-	-		-
	2	-	-	1		1
					Mean	1

Appendix B Table 4

Experiment 4: The effect of anthelmintic treatment on the response to reinfection three weeks later.

Groups O, P and Q received a primary infection of 10 L_3 and 38 days later all four groups were given 3000 L_3. Three weeks before the challenge infection Groups P and Q received anthelmintic treatment.

Individual worm burdens on day four after challenge infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
O	1	1906	3	2	1911
	2	310	1	1	312
	3	1996	1	1	1998
	4	1740	2	1	1743
	5	1120	2	2	1124
	6	1540	1	1	1542
P	1	1504	-	-	1504
	2	1970	-	-	1970
	3	706	-	-	706
	4	2272	-	-	2272
	5	2178	-	-	2178
	6	2186	-	-	2186
Q	1	2270	-	-	2270
	2	2656	-	-	2656
	3	2244	-	-	2244
	4	2284	-	-	2284
	5	2216	-	-	2216
	6	2308	-	-	2308
R	1	2466	-	-	2466
	2	2394	-	-	2394
	3	2372	-	-	2372
	4	2498	-	-	2498
	5	2070	-	-	2070

Contd.

Individual worm burdens on day seven after challenge.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
O	1	0	4	16	20
	2	0	28	100	128
	3	2	58	316	376
	4	0	14	110	124
	5	0	12	110	122
P	1	0	12	104	116
	2	0	4	28	32
	3	8	0	8	16
	4	0	38	330	368
	5	0	2	16	18
	6	0	14	120	134
Q	1	0	6	72	78
	2	4	12	56	72
	3	0	2	12	14
	4	0	0	4	4
	5	0	46	304	350
	6	4	14	54	72
R	1	10	1290	500	1800
	2	6	1600	576	2182
	3	16	1204	532	1752
	4	2	1232	492	1726
	5	6	1094	496	1596

Contd.

Individual worm burdens on day 10 after challenge.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
O	1	0	0	0	0
	2	0	0	0	0
	3	0	0	1	1
	4	0	0	4	4
	5	0	0	2	2
P	1	0	0	0	0
	2	0	0	0	0
	3	0	1	18	19
	4	0	0	0	0
	5	0	0	0	0
Q	1	0	0	2	2
	2	0	0	2	2
	3	0	0	1	1
	4	3	5	43	51
	5	0	0	1	1
R	1	0	1014	450	1464
	2	0	1124	572	1696
	3	0	834	430	1264
	4	4	512	354	866
	5	0	880	438	1318

Contd.

Individual worm burdens of Group S rats which received only the primary infection.

Days after primary infection	Rat No.	Larval stages	Adults		Total
			Females	Males	
17	1	0	3	2	5
	2	0	1	3	4
	3	0	2	1	3
38	1	0	2	3	5
	2	0	3	2	5
	3	0	3	1	4
42 (4)*	1	0	2	1	3
	2	0	2	3	5
	3	0	1	1	2
45 (7)*	1	0	2	3	5
	2	0	2	0	2
	3	0	2	0	2
48 (10)*	1	0	2	0	2
	2	0	1	3	4
	3	0	1	1	2
	4	0	2	4	6

* Days after challenge

Appendix B Table 5

Experiment 5: First experiment to study the immunomodulatory effect of anthelmintics.

Groups T and U received 3000 L_3 and one week before the infection Group T received LEV treatment.

Individual worm burdens four days after infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
T	1	284	0	0	284
	2	840	0	0	840
	3	288	0	0	288
	4	669	0	0	669
	5	703	0	0	703
U	1	402	0	0	402
	2	466	0	0	466
	3	605	0	0	605
	4	729	0	0	729
	5	506	0	0	506

Individual worm burdens on day 10 after infection

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
T	1	3	450	252	705
	2	1	116	103	220
	3	5	386	214	605
	4	2	278	223	503
	5	6	300	298	604
	6	3	435	285	723
U	1	4	332	216	552
	2	1	469	256	726
	3	0	266	183	449
	4	20	591	383	994
	5	9	324	259	592
	6	5	645	381	1031

Contd.

Individual worm burdens on day 14 after infection.

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
T	1	1	2	27	30
	2	1	2	32	35
	3	0	3	26	29
	4	1	11	57	69
	5	0	3	54	57
U	1	2	19	81	102
	2	2	34	95	131
	3	8	134	207	349
	4	4	5	21	30
	5	2	145	146	293

Appendix B Table 6

Experiment 6: Second experiment to study the immunomodulatory effect of anthelmintics.

Groups V, W and X received 3000 L_3 and one week before the infection Groups V and W received anthelmintic treatment.

Individual worm burdens four days after infection

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
V	1	1430	0	0	1430
	2	1178	0	0	1178
	3	899	0	0	899
	4	1138	0	0	1138
	5	760	0	0	760
W	1	1230	0	0	1230
	2	1136	0	0	1136
	3	998	0	0	998
	4	700	0	0	700
	5	1105	0	0	1105
X	1	1336	0	0	1336
	2	765	0	0	765
	3	994	0	0	994
	4	1104	0	0	1104
	5	1236	0	0	1236

Contd.

Individual worm burdens 10 days after infection

Group	Rat No.	Larval stages	Adults		Total
			Females	Males	
V	1	0	352	349	701
	2	0	462	520	982
	3	0	495	512	1007
	4	0	483	412	895
	5	0	450	560	1010
	6	0	263	298	561
W	1	0	511	538	1049
	2	0	551	482	1033
	3	0	477	342	819
	4	0	334	458	792
	5	0	594	590	1184
	6	0	485	352	837
X	1	0	484	380	864
	2	0	474	406	880
	3	0	651	440	1091
	4	0	523	380	903
	5	0	475	490	965
	6	0	514	507	1021

Individual worm burdens 14 days after infection.

V	1	0	1	2	3
	2	0	6	25	31
	3	0	5	15	20
	4	0	23	75	98
	5	0	9	26	35
	6	0	0	1	1
W	1	0	1	1	2
	2	0	4	6	10
	3	0	13	30	43
	4	0	20	35	55
	5	0	8	24	32
	6	0	17	55	72
X	1	0	4	12	16
	2	0	1	3	4
	3	0	6	19	25
	4	0	10	23	33
	5	0	6	40	46
	6	0	28	62	90

Appendix B Table 7

Experiment 7: Do anthelmintics have immunomodulatory properties when sensitising and challenge infections are adult worms transplanted into the duodenum?

Groups A, B and C received a primary infection of 20 D_5 worms and 19 days later Groups A, B, C and D received 600 D_5 worms. Twelve days after the primary infection Groups B and C received anthelmintic treatment.

Individual worm burdens five days after the challenge infection.

Group	Rat No.	Adults		Total
		Females	Males	
A	1	92	55	147
	2	54	33	87
	3	30	22	52
	4	63	59	122
	5	60	63	123
	6	26	29	55
	7	84	75	159
B	1	98	61	159
	2	56	26	82
	3	87	47	134
	4	28	45	73
	5	11	6	17
	6	37	39	76
	7	41	32	73
C	1	14	8	22
	2	40	26	66
	3	74	68	142
	4	13	20	33
	5	88	74	162
	6	37	48	85
	7	59	51	110
D	1	161	74	235
	2	135	66	201
	3	168	58	226
	4	219	104	323
	5	128	78	206
	6	72	34	106
	7	113	76	189

Contd.

Individual worm burdens of Group E rats which received only the primary infection of 20 D_5 worms.

Days after primary infection	Rat No.	Adults Females	Adults Males	Total
12	1	9	8	17
	2	6	2	8
	3	3	4	7
19	1	1	3	4
	2	2	4	6
	3	6	5	11
24 (5)*	1	5	5	10
	2	1	1	2
	3	3	4	7
	4	4	1	5
	5	6	8	14
	6	6	6	12
	7	1	6	7

* Days after challenge infection.

Appendix B Table 9

Experiment 8: Does a challenge infection of transplanted adult worms evade part of the immune response?

Groups A, B, C, D and E received a primary infection of 10 L_3 and 24 days later all six groups received 1500 D_5 worms. Groups B, C, D and E received anthelmintic treatment on day 17, 7, 4 and 1 respectively.

Individual worm burdens seven days after the challenge infection.

Group	Rat No.	Adults		Total
		Females	Males	
A	1	0	0	0
	2	6	7	13
	3	25	22	47
	4	21	15	36
	5	5	3	8
B	1	5	2	7
	2	28	25	53
	3	1	2	3
	4	8	12	20
	5	9	11	20
C	1	41	36	77
	2	7	9	16
	3	37	36	73
	4	2	2	4
	5	0	0	0
D	1	18	9	27
	2	37	24	61
	3	2	0	2
	4	3	0	3
	5	0	0	0
	6	9	8	17
E	1	63	45	108
	2	48	46	94
	3	88	51	139
	4	0	0	0
	5	3	4	7
	6	2	1	3
F	1	268	171	439
	2	172	140	312
	3	223	221	444
	4	292	178	470
	5	301	157	458

Contd.

Individual worm burdens of rats from Groups A, B and C necropsied after the primary infection.

Group	Rat No.	Necropsy - Days after primary infection	Adults		Total
			Females	Males	
C	1	7	3	1	4
	2		2	2	4
	3		3	3	6
	4		2	3	5
	5		3	2	5
B	1	17	2	1	3
	2		4	3	7
	3		2	2	4
	4		3	2	5
	5		3	2	5
A	1	24	1	2	3
	2		3	2	5
	3		2	3	5

Appendix B Table 10

Experiment 9: Is a sensitising infection of transferred adult worms a relatively poor immunogenic stimulus compared with that provided by a larval infection.

Groups G, H and I received a primary infection of 300 D_5 worms and 24 days later Groups H and J received 800 D_5 worms while Groups I and K were given 3000 L_3.

Individual worm burdens seven days after the challenge infection.

Group	Rat No.	Adults		Total
		Females	Males	
H	1	0	4	4
	2	14	21	35
	3	12	15	27
	4	5	6	11
	5	7	7	14
I	1	2	2	4
	2	0	1	1
	3	2	0	2
	4	12	8	20
	5	17	8	25
J	1	120	73	193
	2	86	64	150
	3	78	57	135
	4	87	70	157
	5	108	77	185
	6	89	67	156
K	1	873	533	1406
	2	621	416	1037
	3	789	338	1127
	4	925	501	1426
	5	701	421	1122
	6	786	350	1136

Individual worm burdens of rats from Group A on day 24 after primary infection.

A	1	1	1	2
	2	0	2	2
	3	2	9	11
	4	3	5	8

Appendix B Table 11

Experiment 10: The response of immunised rats to low levels of challenge with N. brasiliensis

Groups L, O and R received a primary infection of 10 L_3 while Groups M, P and S received 250 D_5 worms at the same time. Twenty-four days later Groups L, M and N were given 300 L_3 while Groups O, P and Q were given 30 L_3.

Individual worm burdens seven days after the challenge infection.

Group	Rat No.	Adults		Total
		Females	Males	
L	1	9	2	11
	2	6	20	26
	3	11	6	17
	4	9	8	17
	5	8	2	10
M	1	4	4	8
	2	8	5	13
	3	3	3	6
	4	16	10	26
	5	6	3	9
	6	15	9	24
N	1	35	42	77
	2	51	52	103
	3	48	43	91
	4	55	52	107
	5	64	47	111
O	1	7	7	14
	2	9	10	19
	3	4	7	11
	4	8	6	14
	5	4	5	9
P	1	0	0	0
	2	1	2	3
	3	1	1	2
	4	2	4	6
	5	3	2	5
	6	1	3	4
Q	1	9	7	16
	2	8	9	17
	3	6	7	13
	4	8	8	16
	5	8	10	18

Contd.

Individual worm burdens of rats on day 10 after challenge infection

Group	Rat No.	Adults		Total
		Females	Males	
L	1	6	6	12
	2	18	20	38
	3	7	10	17
	4	7	9	16
	5	8	12	20
M	1	1	1	2
	2	7	9	16
	3	8	12	20
	4	4	2	6
	5	8	10	18
N	1	46	33	79
	2	33	31	64
	3	39	32	71
	4	71	56	127
	5	32	29	61
O	1	3	6	9
	2	6	10	16
	3	8	9	17
	4	6	8	14
	5	9	9	18
P	1	0	1	1
	2	3	4	7
	3	3	4	7
	4	1	1	2
	5	1	4	5
Q	1	10	7	17
	2	8	12	20
	3	7	7	14
	4	9	9	18
	5	10	8	18

Contd.

Individual worm burdens of rats from Groups R and S which received the primary infection only.

Group	Rat No.	Days after primary infection	Adults		Total
			Females	Males	
R	1	24	1	1	2
	2		1	2	3
	3		2	3	5
	4		3	2	5
	5		3	1	4
S	1	24	3	4	7
	2		6	15	21
	3		2	2	4
	4		3	6	9
	5		2	2	4
R	1	31(7)*	1	2	3
	2		2	1	3
S	1	31(7)*	6	3	9
	2		1	3	4
	3		10	5	15
R	1	34(10)*	1	1	2
	2		2	2	4
S	1	34(10)*	2	4	6
	2		1	4	5
	3		2	3	5

* Days post-challenge.

Appendix B

Experiment 1: First experimental attempt to produce a moderate degree of immunity to *N. brasiliensis* with low sensitising infections.

Groups	Primary infection Day 0	Mean group faecal egg counts (epg) 6 - 24 days after primary infection									
		D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14	D 24
A	50 L_3	1250	1100	2350	450	175	50	50	0	0	50
B	100 L_3	2150	2650	3700	1000	500	0	<50	0	0	50
C	500 L_3	13450	12900	8500	2400	550	0	0	0	0	100
D	-	0	0	0	0	0	0	0	0	0	0

Groups	Primary infection Day 0	Challenge Infection Day 24	Mean group faecal egg counts (epg) 4-14 days after challenge									
			D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
A	50 L_3	3000 L_3	100	50	750	50	75	25	50	0	100	0	0
B	100 L_3	3000 L_3	250	100	100	75	50	0	0	0	0	0	0
C	500 L_3	3000 L_3	0	0	25	<50	225	25	0	0	0	0	0
D	-	3000 L_3	0	0	65700	124500	73350	72900	141750	161375	9050	550	0

Appendix B

Experiment 2: Second experimental attempt to produce a moderate degree of immunity to *N. brasiliensis* with low sensitising infections.

Groups	Primary infection	Mean group faecal egg counts (epg) after primary infection																
	Day 0	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17
E	100 L_3	0	0	0	0	0	250	2375	3475	2425	3000	4175	1525	1150	675	100	225	175	300
F	50 L_3	0	0	0	0	0	100	975	1275	1200	1750	2200	800	725	400	175	200	150	225
G	25 L_3	0	0	0	0	0	0	425	900	625	475	900	1100	350	300	300	325	150	125
H	10 L_3	0	0	0	0	0	0	175	700	275	300	225	400	175	175	150	100	75	150
I	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Groups	Primary infection	Challenge infection	Mean group faecal egg counts (epg) after challenge infection									
	Day 0	Day 24	D 0	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10
E	100 L_3	3000 L_3	0	<25	<25	25	0	75	150	100	100	100	50
F	50 L_3	3000 L_3	100	25	0	0	0	0	25	50	0	0	0
G	25 L_3	3000 L_3	50	25	0	100	0	0	175	150	50	50	0
H	10 L_3	3000 L_3	25	0	25	75	125	25	700	125	0	0	0
I	-	3000 L_3	0	0	0	0	0	50	13750	57900	39900	40000	6450

Appendix B

Experiment 4: The effect of anthelmintic treatment on the response to reinfection three weeks later.

Groups	Primary infection Day 0	Anthelmintic treatment Day 17	Mean group faecal egg counts (epg) after primary infection																				
			D0	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D21	D22	D23	D28	D35	D38
O	10 L_3	-	0	0	0	275	350	550	250	225	275	75	75	50	75	25	100	50	100	25	75	50	100
P	10 L_3	LEV	0	0	25	275	400	500	450	250	100	200	100	75	50	50	50	0	0	0	0	0	0
Q	10 L_3	TBZ	0	0	75	250	475	475	325	325	150	125	125	125	75	75	0	0	0	0	0	0	0
R	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	10 L_3	-	0	0	125	225	250	200	475	275	300	100	175	50	50	50	50	0	0	100	50	125	75

Groups	Challenge infection D38	Mean group faecal egg counts (epg) after challenge infection								
		D 0	D 1	D 4	D 5	D 6	D 7	D 8	D 9	D 10
O	3000 L_3	100	0	75	0	75	400	150	0	0
P	3000 L_3	0	0	0	25	3000	425	50	50	0
Q	3000 L_3	0	0	0	75	1650	750	50	0	0
R	3000 L_3	0	0	0	550	51600	76650	58700	4400	500
S	-	75	50	0	50	0	0	50	0	0

Appendix B

Experiment 5: First experiment to study the immunomodulatory effect of anthelmintics.

Groups	Anthelmintic treatment D -7	Infection D 0	Mean group faecal egg counts (epg) after infection													
			D -7	D 0	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 10	D 11	D 12	D 13	D 14
T	LEV	3000 L_3	0	0	0	0	0	0	0	9250	18100	25500	1250	50	50	100
U	-	3000 L_3	0	0	0	0	0	0	0	7500	23550	22400	13800	6200	100	150

Appendix B

Experiment 6: Second experiment to study the immunomodulatory effect of anthelmintics.

Groups	Anthelmintic treatment Day -7	Infection Day 0	Mean group faecal egg counts (epg) after infection															
			D-7	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
V	LEV	3000 L_3	0	0	0	0	0	0	0	68400	91500	47000	28900	5600	0	0	0	0
W	TBZ	3000 L_3	0	0	0	0	0	0	0	119000	81400	73100	27400	8400	0	0	0	0
X	-	3000 L_3	0	0	0	0	0	0	0	82700	57700	46800	15500	7700	0	0	0	0

Appendix B

Experiment 7: Do anthelmintics have immunomodulatory properties when sensitising and challenge infections are adult worms transplanted into the duodenum?

Groups	Primary infection D 0	Anthelmintic treatment D 12	Mean group faecal egg counts (epg) after primary infection													
			D 1	D 2	D 5	D 6	D 7	D 8	D 9	D 11	D 12	D 13	D 14	D 15	D 16	D 18	D 19
A	20 worms	-	650	2100	600	400	150	450	550	300	400	150	350	50	600	<50	100
B	20 worms	LEV	350	850	200	500	50	150	300	100	150	100	0	0	0	0	0
C	20 worms	TBZ	250	850	300	600	300	250	300	100	150	200	0	0	0	0	0
D	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	20 worms	-	100	950	450	450	450	250	450	200	350	150	250	150	50	50	200

Groups	Challenge infection D 19	Mean group faecal egg counts (epg) after challenge infection				
		D 1	D 2	D 3	D 4	D 5
A	600 worms	2000	900	1100	1200	550
B	600 worms	1250	800	250	600	250
C	600 worms	1050	1150	300	150	50
D	600 worms	1400	5400	5300	9800	3400
E	-	100	50	200	150	50

Appendix B

Experiment 8: Does a challenge infection of transplanted adult worms evade part of the immune response?

Groups A, B, C, D and E received 10 L_3 on day 0 and 24 days later all six groups received 1500 D_5 worms.

Groups B, C, D and E received anthelmintic treatment on day 17, 7, 4 and 1 respectively.

Groups	Mean group faecal egg counts (epg) after primary infection																				
	D0	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24
A	0	0	300	600	150	350	350	150	50	100	100	150	150	150	100	100	50	50	100	50	50
B	0	0	50	500	550	500	750	150	150	300	200	250	50	100	0	0	0	0	0	0	0
C	0	0	0	400	450	150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	<50	100	50	0	0	0	0	0	0	0	0	0	0	0	0	0	50	50
E	0	0	0	150	<50	<50	<50	0	0	0	0	0	0	0	50	0	50	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Contd.

Groups	Mean group faecal egg counts (epg) after challenge infection						
	D 1	D 2	D 3	D 4	D 5	D 6	D 7
A	4000	300	500	350	0	0	0
B	2100	200	100	150	500	150	50
C	2200	3500	850	300	600	0	50
D	1450	650	250	50	0	0	0
E	1800	1700	500	100	150	0	0
F	2500	5500	10000	23400	17300	3200	2600

Appendix B

Experiment 9: Is a sensitising infection of transferred adult worms a relatively poor immunogenic stimulus compared with that provided by a larval infection?

Groups G, H and I received 300 D_5 worms on day 0 and 24 days later Groups H and J received 800 D_5 worms while Groups I and K were given 3000 L_3.

Groups	Mean group faecal egg counts (epg) after primary infection																						
	D0	D1	D2	D3	D4	D5	D6	D7	D8	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24
G	0	750	3600	3100	5700	1700	2100	1200	800	100	0	0	0	0	0	0	0	0	0	50	0	0	0
H	0	250	3300	1900	4200	2600	2300	1150	1050	100	0	50	<50	100	0	0	0	0	0	50	150	50	50
I	0	850	2000	6300	11200	4800	4400	1300	600	50	0	0	100	<50	0	0	0	0	0	0	100	50	100
J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Groups	Mean group faecal egg counts (epg) after challenge infection						
	D 1	D 2	D 3	D 4	D 5	D 6	D 7
H	150	0	0	100	50	0	0
I	50	0	0	0	0	0	0
J	1200	3400	6000	15500	2000	4000	4700
K	0	0	0	0	45000	15900	28900

Appendix B

Experiment 10: The response of immunised rats to low levels of challenge with N. brasiliensis.

Groups L, O and R received 10 L_3 on day 0 while Groups M, P and S received 250 D_5 worms at the same time. Twenty four days later Groups L, M and N were given 300 L_3 while Groups O, P and Q were given 30 L_3.

Groups	Mean group faecal egg counts (epg) after primary infection																				
	D0	D1	D2	D3	D4	D5	D6	D7	D8	D9	D12	D13	D14	D15	D16	D19	D20	D21	D22	D23	D24
L	0	0	0	0	0	0	200	150	350	300	100	350	100	100	0	50	50	0	0	0	0
M	0	1500	1900	3300	2750	1750	5000	250	400	350	0	0	0	0	0	0	0	0	0	0	0
N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0	0	0	0	0	0	100	150	250	50	0	0	0	200	50	0	0	0	0	0	0
P	0	1100	900	1200	1100	1650	1600	1150	700	350	0	0	0	0	0	0	0	150	0	0	0
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	0	0	100	250	400	50	150	300	0	<50	150	0	50	0	0	0	0
S	0	500	2000	800	1200	2400	4200	1450	1650	600	0	0	0	100	0	0	0	50	0	0	0

Contd.

Groups	Mean group faecal egg counts (epg) after challenge infection								
	D 1	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10
L	0	0	0	<50	200	150	50	50	150
M	0	0	0	0	50	250	600	50	50
N	0	0	0	50	3300	3950	3150	1200	500
O	0	0	0	50	100	50	100	50	50
P	0	0	0	0	0	100	100	0	100
Q	0	0	0	0	300	1750	1550	2800	650
R	0	0	<50	<50	0	300	50	50	<50
S	0	50	0	0	50	200	50	50	0