SOME PROBLEMS ASSOCIATED WITH THE VACCINATION OF RUMINANTS AGAINST HELMINTH INFECTIONS.

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by

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GENERAL INTRODUCTION.

Helminth infections of farm livestock present one of the major obstacles to the improvement of animal production so necessary to alleviate the worldwide shortage of protein.

The precise extent to which helminths affect productivity is not readily determined since helminth diseases are generally chronic in nature with low mortality and high morbidity and such an effect is difficult to assess economically. Heavy infections with some helminths do, of course, produce acute disease with a high mortality and readily calculable economic losses.

One attempt to calculate the losses due to helminth infections of farm animals on a national scale was made by the United States Agricultural Research Service[1]; the estimated amount of such losses in the U.S.A. during 1954 was 227,672,000 dollars. In a further discussion on the data provided by this survey Foster[2] suggested that this estimate was too low and postulated that parasitic infections embracing both internal helminths and external arthropods caused 40% of the total animal disease in farms in the U.S.A. with losses amounting to 1 billion dollars annually.

In the United Kingdom no attempt has been made to assess the national debt due to helminth diseases, although efforts have been made to determine losses due to individual helminths. For example, in the case of the lung nematode Dictyocaulus viviparus, which causes

parasitic bronchitis of cattle, it has been calculated that mild, moderate or severe disease in yearling calves may result in a production loss of £9, £17 and £27 per animal respectively[3]. These figures do not include losses due to deaths and are therefore a conservative estimate. Since parasitic bronchitis is endemic in Britain and 4.5 million calves of both dairy and beef breeds are reared annually[4] the recurrent annual losses due to this disease are considerable.

Another important disease in Britain affecting mainly dairy calves is bovine ostertagiasis caused by the stomach worm Ostertagia ostertagi. This parasite exerts its pathogenic effect by altering the secretory cell composition of the gastric glands so that the pH in the abomasum is reduced with resultant impaired digestion, and poor food conversion rates and weight gains. The extent of the growth lag may be considerable and in dairy calves only lightly infected with O. ostertagi mean body weight gains may be 60 kg greater than those of heavily infected animals over a grazing period of 3-4 months[5]. In Britain, dairy farming policy is arranged so that replacement female animals calve for the first time at 2 years old when they weigh approximately 500 kg. Since ostertagiasis is endemic on dairy farms, many of the 1 million replacement females reared annually do not achieve a satisfactory liveweight gain so that breeding may be postponed for up to one year.

The liver trematode, Fasciola hepatica is also prevalent in Britain and a recent abattoir survey[6] found that 21% of

cattle livers had lesions of fascioliasis. Since this parasite is known to be responsible for poor weight gains, deficient carcass composition, lowered milk production and condemnation of livers at marketing, the combined effects are calculated to cause annual losses in excess of £50 million pounds.

In many tropical and sub-tropical areas it is universally accepted that the stomach nematode Haemonchus contortus is the major drawback to profitable sheep production. Although an exact figure is not available for the economic waste caused by this parasite, individual studies have shown that it is responsible for impaired weight gains, poor wool growth and sub-optimal breeding performance[7,8].

As information on the extent to which helminth diseases impair the agricultural economy has accumulated strenuous efforts have been made to find efficient measures of controlling them. Unfortunately, complete prevention of infection is extremely difficult for several reasons. First, the free-living infective larval stages of most helminths both free-living and in an intermediate host, are highly resistant and capable of surviving for at least one year on pasture; second, wild animals such as deer, rabbits and hares may act as reservoirs of infection for some helminths; third, mechanical transport or dissemination of free-living stages may occur by various means, such as, human agencies, dipteran flies[9], birds and fungi[10].

Despite these difficulties several methods of control have been developed and by applying these the level of infection can be reduced and economic losses minimised. For example, significant levels of infection can be prevented by grazing animals on newly sown pastures or older pastures not grazed by susceptible hosts for at least one year. Alternatively, grass from these pastures is cut and fed daily to housed stock, so-called "zero grazing" . However, the current pressure on land usage is considerable, and it is unlikely that the entire grazing area on any particular farm will be completely free of helminth ova or larvae; furthermore, since there is no significant age immunity or resistance to many helminths, older animals not exposed in early life may suffer from parasitism during their later and more productive years. The trend therefore has been to expose young stock to a low level of infection which will not severely impair their productivity but allow the development of an acquired immunity.

This theme is illustrated by the technique proposed by Michel[11] for the control of bovine ostertagiasis which utilises a combination of rotational grazing and anthelmintic therapy. In this system calves are turned out to graze in the spring (April/May) and are moved to aftermath by mid-July, this move being accompanied by an anthelmintic treatment; the animals then graze on aftermath until housed at the onset of winter in October or November. The parasitological and immunological basis for these measures are that in the

spring the calves ingest the overwintered larval infection on the pasture and Ostertagia eggs appear in the faeces 3-4 weeks later (May or June). However, the climatic conditions prevailing in U.K. prevents these eggs becoming infective larvae until at least mid-july; if the calves are treated with an anthelmintic and moved prior to that time they will avoid the new wave of infection in late July and August. This system has proved satisfactory in the control of ostertagiasis in that calves acquire a level of infection to low to impair production but sufficient to stimulate resistance to infection in subsequent years.

Another grazing system designed to control bovine ostertagiasis and possibly dictyocauliasis was described by Leaver[12]. In this system susceptible calves are rotationally grazed in permanent paddocks followed by pregnant heifers or cows, which are presumed to be immune by previous contact with the helminth. The adult animals act as "vacuum cleaners" removing ova and larvae from the herbage and in this way, when calves return to the previously grazed paddocks, only a low larval challenge remains. The success of the system is dependent on the ratio of immune animals to susceptibles and must not exceed 1:6[13]. This method has given good control of ostertagiasis but failed adequately to control dictyocauliasis.

Another approach sometimes used is mixed grazing of different animal species, either on the same pasture or alternatively on adjacent pastures[14,15]. This has the

disadvantage that some parasites are capable of infecting different hosts, e.g. Trichostrongylus axei and F. hepatica may infect sheep, cattle and horses, while H. contortus may infect all grazing ruminants.

Since the advent of the first broad spectrum anthelmintic, namely thiabendazole in the early 1960's[16] a succession of new anthelmintics effective against most of the common helminths of farm animals have been discovered, e.g. tetramisole[17]; morantel tartrate[18]; diamphenethide[19] and the successful treatment of gastrointestinal helminthiasis is now feasible. In current practice, the optimal application of these new anthelmintics is based on their prophylactic or strategic use rather than a therapeutic role and successful prophylaxis of several helminth diseases has been achieved. However, there are several drawbacks to the control of helminthiasis by strategic anthelmintic therapy. Thus, new compounds are expensive as is regular handling of livestock for treatment. Also, since these drugs are excreted in the milk and their metabolic products deposited in body tissues, legislation has decreed that neither milk nor carcass may be sold for human consumption for a certain period after treatment. In addition, some helminths have unfortunately developed resistance to the new anthelmintics, e.g. resistant strains of H. contortus against thiabendazole have been reported by Drudge, Szanto, Wyant and Elam[20] and Smeal, Gough, Jackson and Hotson[21] while Round, Simpson, Haselden, Glendinning and Baskerville[22] have reported resistance to thiabendazole and mebendazole among the small strongyles of horses.

A more attractive approach to the control of helminthiasis is undoubtedly the immunological one and considerable work has been carried out in the past two decades in an attempt to successfully immunise animals against helminths, particularly using attenuated larvae as the immunising agents. The first attempt to attenuate helminth larvae was made by Tyzzer and Honeij[23] who used ionising radiation to so attenuate encysted larvae of Trichinella spiralis that when the latter were fed to mice they failed to become established. However, this investigation attracted only minor interest for the next 4 decades[24,25,26,27], until Jarrett, Jennings, McIntyre, Mulligan and Urquhart[28] carried out their studies on the immunisation of cattle against D. viviparus using larvae attenuated by X-rays. In their first series of experiments using a single immunising dose the protection achieved against an experimental challenge infection or a natural field challenge was 80%[29]. When two immunising doses of attenuated larvae were given at an approximate interval of one month the protection obtained against both experimental and field challenge was excellent being in the region of 94-100%[30,31,32]. The outcome of this research was the first commercial production of a vaccine for use against helminths.

Since then several other parasites have been attenuated in the same manner and have been used to immunise a range of hosts with a varying degree of success. For example, in sheep, both Jarrett, Jennings,

McIntyre and Sharp[33] and Mulligan, Gordon, Stewart and Wagland[34] found that attenuated Trichostrongylus colubriformis larvae gave good protection against experimental challenge, while attenuated larvae of H. contortus provided an excellent immunity in 7 month old sheep also to an experimental challenge[35,36]. Later, however, Urquhart, Jarrett, Jennings, McIntyre, Mulligan and Sharp[37] and Urquhart, Jarrett, Jennings, McIntyre and Mulligan[38] failed to achieve protection of younger sheep, i.e. 2-3 month old lambs using X-irradiated H. contortus larvae. In Yugoslavia Jovanovic, Sokolic, Movsesijan and Cuperlovic[39] successfully immunised lambs aged 4 months or older against sheep lungworm D. filaria; successful vaccination against this species has also been reported by Tewari, Dhar and Singh[40].

In cattle good protection was achieved against Cysticercus bovis, the intermediate stage of the human tapeworm Taenia saginata using X-irradiated oncospheres[41]. In the same host Ross, Armour Hart and Lee[42] found that X-irradiated Haemonchus spp. infective larvae when administered to calves, conferred 60% protection against an experimental challenge; however, against natural challenge protection was not observed (Armour, personal communication). Using X-irradiated O. ostertagi infective larvae Armour[43], Burger, Eckert, Chevalier, Rahman and Konigsman[44] and Burger and Pfeiffer[45] failed to achieve a significant immunity to either an experimental or field challenge.

In the dog, X-irradiated larvae of the hookworm Uncinaria stenocephala[46,47] and Ancylostoma caninum[48,49] stimulated an excellent immunity against experimental challenge and in poultry Varga[50] successfully immunised against the tracheal nematode Syngamus trachea using X-irradiated infective larvae. Finally, recent studies in foals by Duncan[51] and Mansley[52] demonstrated that a partial immunity could be obtained against Strongylus vulgaris again using X-irradiated larvae.

Although immunity could be induced in various degrees against these helminths under experimental conditions, until now, the only vaccines commercially available are those against D. viviparus in Europe, D. filaria in Eastern Europe, A. caninum in U.S.A. and S. trachea in Hungary. Considering the extent of the research effort in the field of attenuated helminth larval vaccines in the past two decades, the fact that only these four are being used commercially is disappointing.

There are several possible obstacles to the immunisation of animals with irradiated helminth vaccines, of which the inability to respond successfully to a helminth antigen during the first few weeks of life is probably the greatest. This has been demonstrated with H. contortus in lambs by Manton, Peacock, Poynter, Silverman and Terry[53] and Urquhart et al.,[37,38] and in Nippostrongylus brasiliensis in rats by Jarrett[54], and even the most successful of the helminth vaccines yet

developed, namely the D. viviparus irradiated larval vaccine, is only recommended for calves of at least 8 weeks old. The reason for this poor immunological response in the young is not known but may be due to immunological immaturity, acquired immunological unresponsiveness in endemic areas, or, in the case of suckling animals, the blocking effect of maternal antibodies. It may also be significant that successful immunisation against helminths has been achieved with parasites which have a somatic migration while relatively poor immunisation has been obtained with helminths which live in the intestine without prior migration and possibly limited contact with the host's immune defences.

The experiments described in this thesis were designed to investigate some of the problems pertaining to vaccination with one helminth which exhibits somatic migration, namely D. viviparus in calves and two helminths which do not migrate in the host, O. ostertagi in calves and H. contortus in sheep. In particular the influence of age, suckling and acquired immunological unresponsiveness have been investigated.

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MATERIALS AND METHODS.

Rearing of Parasite-free Animals

Calves used in these experiments were males of the Ayrshire breed and were purchased when they were a few days old from various farms in the West of Scotland. They were fed colostrum for the first 24 hours on the farm and subsequently transported to the Veterinary School where their individual gamma-globulin levels were measured by the zinc sulphate turbidity test. If the immunoglobulin levels were satisfactory (more than 10 turbidity units) the calves were retained and according to the experimental design either fed milk substitute* twice daily or suckled on foster mothers. Four suckled calves were allocated to each foster mother and their calves were allowed to feed 3 times a day at 7 a.m., 12 noon and 6 p.m. for a period of 1 hour. All calves had hay and water ad libitum, and were offered concentrate from the third week in an increasing quantity up to two pounds twice daily; weaning took place at 6-8 weeks of age.

Day-old Scottish Blackface lambs were obtained from a nearby farm and bottle-fed indoors with milk substitute. Again water and hay were available from the first week and concentrates were offered from the third week. The lambs were weaned at 6 weeks of age.

All animals were housed in concrete pens which were thoroughly cleaned with water twice per week and were provided with fresh straw bedding daily.

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* Denkavit, Rank, Hovis MacDougal, Mid Calder, Scotland.

Weighing

All animals were weighed weekly on an Avery cattle scale with the exception of those at pasture, which were weighed monthly. Weighing was carried out in the morning 2-3 hours after feeding and always at the same time to eliminate a possible source of variation.

Clinical Observations

All animals were examined daily and their food intake and general condition noted. In the calf lungworm studies the respiration rate and presence or absence of a cough were recorded, prior to a full examination of the respiratory system which included auscultation of both lungs. The details of any abnormal respiratory signs including their nature, time of onset and severity were also recorded.

In the studies of ostertagiasis in calves and H. contortus infection in sheep a record was kept of the variation in faecal consistency. An arbitrary classification of faeces was made as follows:

+	=	soft faeces
++	=	semi-fluid faeces
+++	=	fluid faeces

Blood Analysis

Collection and storage of samples

Blood samples were collected directly from the jugular vein once or twice a week into heparinised evacuated glass containers (Vacutainer No. 3204, Becton, Dickinson & Co., Rutherford, N.J., U.S.A.). Samples obtained in non-heparinised vacutainers were allowed to stand overnight on the laboratory

bench. Serum separated from these clotted samples was centrifuged at room temperature for 20 minutes at 2,000 r.p.m. in an MSE centrifuge (Measuring Scientific Equipment, London, England) and then pipetted into polyethylene tubes and immediately stored at -20°C.

Packed cell volume (PCV)

The packed cell volume was measured by the micro-haematocrit method[1]. Capillary tubes (Gelman-Hawksley Ltd., Lancing, Sussex, England) were filled with blood and sealed at one extremity by heat and then centrifuged for 5 minutes in a microhaematocrit centrifuge (Hawksley & Co. Ltd., London, England). The PCV was determined using the Hawksley Microhaematocrit Reader.

Haemoglobin concentration (Hb)

The estimation of haemoglobin concentration in grams per 100 ml of blood was carried out using the oxyhaemoglobin method of Dacie and Lewis[2]. An 0.04% solution of ammonium hydroxide was used to make a 1:200 dilution of blood. After thorough mixing the tubes were allowed to stand for 10-20 minutes before being read in an EEl colorimeter (Evans Electroselenium Ltd., Harlow, England) using a yellow green filter (Ilford No. 625). The results were calculated by reference to a chart prepared from readings of standard haemoglobin solutions.

Total red blood cell counts (RBC)

Red blood cell counts were determined using an electronic particle counter (Coulter Model "D", Coulter Industrial Sales Co., Elmhurst, 111, U.S.A.).

Haemoglobin Typing

The 3 haemoglobin types found in sheep can be separated by electrophoresis. Although several electrophoretic methods are available, the cellulose acetate was selected for its simplicity.

Cellulose acetate strips* were saturated with tris-borate buffer at a pH of 9 (Tris(Hydroxymethyl amino methane) 16.1 g, Disodium EDTA 1.56 g, Boric acid 0.93 g and distilled water 1,000 ml). The paper strip was lightly blotted to remove any excess of liquid and placed in an electrophoresis tank*. The tank was previously half filled with barbitone buffer at a pH of 8.5 (Barbitone 1.84 g, Sodium barbitone 10.3 g and distilled water 1,000 ml). Bloods from individual sheep were haemolysed by mixing one drop with an equal amount of distilled water on an applicator plate. After thorough mixing 10 samples were transferred using an applicator to the cellulose acetate paper in the electrophoresis tank at about 2 cm from the cathode. A lid was applied to the tank and the samples subjected to 150 volts for 30 minutes. The strips were than transferred to a tray containing 5% aqueous solution of trichloroacetic acid (TCA) for 5 minutes, and stained with 0.2% Ponceau S** in 3% aqueous TCA for 5 minutes. Finally each strip was washed in 5% aqueous acetic acid until the background acquired a white colour.

The different Hb types were easily recognised by their different rates of migration. HbA migrated the farthest and HbB the least, with HbAB occupying an intermediate position.

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* Shandon Instruments, Camberley, Surrey.
**G.T. Guss Ltd., London.

Plasma pepsinogen

Plasma pepsinogen was estimated by a method essentially similar to that of Edwards, Jepson and Wood[3] in which plasma was incubated at 37°C for 24 hours at a pH of 2.0 with a bovine serum albumin substrate (Bovine Albumin Fraction V., British Drug House, Poole, England). The liberated tyrosine, non-precipitable with trichloroacetic acid, was estimated with Folin-Ciocalteau reagent (BDH) and read in a spectophotometer (Unicam, Cambridge, England). The enzyme activity was expressed as milli-units (mU) of tyrosine.

Serological Techniques

Passive haemagglutination

The procedure followed was described by Herbert[4]. Sheep red blood cells (SRBC) were obtained from the jugular vein and aged for 3 days in Alsever's solution (Dextrose 2.05 g, Sodium citrate 0.80 g, Sodium chloride 0.42 g, Distilled water 100 ml). The SRBC were then washed 3 times with approximately 10 volumes of phosphate buffered saline PBS (NaCl 36 g, Na2HPO4 (anhydrous) 7.4 g, KH2PO4 (anhydrous) 2.15 g) centrifugating each time at 750 g for 15 minutes.

Tanning procedure

After washing the cells were treated with tannic acid according to the method described by Herbert[4]. SRBC and tannic acid were reacted in the following proportions: 0.6 ml packed SRBC were mixed with 10 ml tannic acid solution (0.1 mg/ml) and incubated for 15 minutes at 37°C. The cells were then centrifuged for 5 minutes at 750 g and

washed once in 20 ml PBS and finally resuspended in 10 ml PBS.

Coating procedure

The antigen was prepared from 2,500 adult D. viviparus which were completely homogenised (Silverson Machines (Sales) Ltd., London, England) in 20 ml PBS. The homogenate was then centrifuged and only the supernatant used.

After tanning the cells were again washed in PBS and 10 ml of the antigen preparation was mixed with 2 ml packed, tanned SRBC and gently agitated for 45 minutes at room temperature. After centrifugation and removal of the antigen solution, the coated cells were washed 3 times in PBS containing 1% normal rabbit serum which had been previously inactivated and absorbed with tanned SRBC. The antigen coated cells were finally resuspended as a 1% suspension in the PBS-1% normal rabbit serum.

Then 0.5 ml of each test sera was absorbed with the equivalent of 0.02 ml packed tanned SRBC for 30 minutes at 37°C, then overnight at 4°C. After centrifugation the absorbed sera were used for the haemagglutination test.

Test

Doubling dilutions of the absorbed test sera were prepared in 25 µl PBS in microtitre plates (Biocult Laboratories Ltd., Paisley, Scotland). To each well was added 25 µl of a 2% suspension of washed antigen coated tanned SRBC. The plates were covered and left overnight at room temperature before the haemagglutination

titres were read. The antibody titres were read as being the highest dilution of antiserum giving complete agglutination.

Zinc sulphate turbidity test

In newly purchased calves the immunoglobulin levels were determined by the method of McEwan, Fisher, Selman and Penhale[5].

A solution of zinc sulphate (208 mgm/litre) was prepared in a volumetric flask using carbon dioxide-free distilled water. Two matched colorimeter tubes were then taken and 6 ml of distilled water were placed into the first (control) and 6 ml of the zinc sulphate solution were placed into the second. An 0.1 ml sample of the serum under test was then delivered into each tube. The tubes were gently shaken and left standing for 30 minutes at room temperature. These tubes were then read using an Ilford blue green filter (No. 623), in an EEL colorimeter previously zeroed with a tube containing only 10 ml distilled water. The turbidity value was then obtained by subtracting the control tube turbidity value from that of the test tube.

Parasitological Techniques

Faecal egg count method

Before each experiment began, all animals were faecal sampled regularly to test for the presence of eggs or larvae of nematodes by Gordon and Whitlock's[6] modification of the McMaster faecal egg counting technique. Three grams

of faeces were mixed with 42 ml of tap water and homogenised using an MSE homogeniser. The resulting mixture was passed through a sieve (Endecotts (Filters) Ltd., London, England) of 60 meshes per inch and the liquid transferred to a 15 ml flat bottomed test tube. After being centrifuged (Measuring Scientific Equipment, London, England) at 2,000 r.p.m. for 3 minutes, the supernatant was decanted and the sediment shaken using a Whirlmixer (Fisons Scientific Apparatus Ltd., Loughborough, Leicestershire). A saturated solution of sodium chloride was then added to the sediment and the tube inverted 6 times to ensure complete mixing. Using a Pasteur pipette, both chambers of a McMaster Counting slide (Hawksley & Sons, London, England) were filled and examined under the microscope. The number of eggs or larvae found in both chambers was multiplied by 50 to give the total number of eggs or larvae per gram of faeces.

Larval culture

Parasite-free animals were infected orally with the appropriate infective larvae (100,000 O. ostertagi to calves and 5,000 H. contortus to lambs). From the 18th day after infection the animals were checked daily and when a sufficiently high number of eggs was detected in the faeces they were fitted with faecal bags and collections were made every 24 hours. The total daily collection of calf faeces was mixed thoroughly with vermiculite (Horticultural vermiculite No. 2 size) until a crumbling mixture was obtained. In the case of sheep faeces it was

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not usually necessary to add vermiculite. Two hundred to three hundred gram samples were then transferred into 1 lb. honey jars with the top lightly screwed down and put into a dark cabinet at a temperature of 20-22°C for about 15 days, this being the time estimated for development of all the larvae to the infective stage (L_3).

Infective larvae were collected by a method essentially similar to that described by Roberts and O'Sullivan[7]. The jars were unscrewed and filled to the brim with lukewarm tap water. After one hour under diffuse light the fluid from the jars was poured through a 60 mesh per inch sieve and then on to a double layer of milk-filters (Cloverleaf No. 9, Johnson & Johnson, Slough, Buckinghamshire, England) placed on top of a Buchner funnel. The larvae trapped in these milk-filters were then transferred to a Baermann apparatus, left overnight and subsequently collected from the neck of the funnel. D. viviparus infective larvae were obtained directly from the laboratory of Allen & Hanburys, Ware, England.

Larval counting technique

The larval counting procedurewas as follows: After thorough mixing to prevent the larvae forming clumps, 20 aliquots were taken using an 0.025 ml pipette and transferred to glass slides for counting. The mean was calculated, and multiplied by 40 to give the number of larvae per ml. Larval doses were prepared in 25 ml

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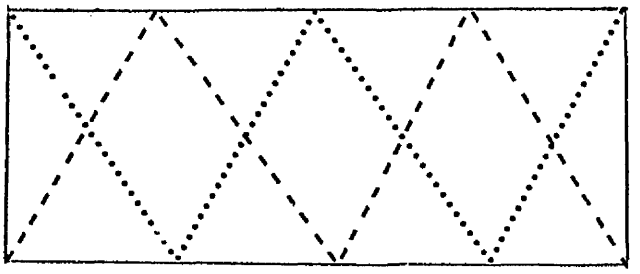
universal bottles and administered orally to the animals taking care that no larvae remained in the bottles. Only freshly collected larvae were used, and the culture from which these were obtained was never more than 2 months old.

Recovery of larvae from pasture

Pasture samples were obtained by a modification of the method described by Parfitt[8] in which the experimental plot was crossed following a pattern shown in the accompanying diagram; 100 samples were collected along both Route 1 and Route 2.



These samples were taken from 4 areas around the feet by removing a small amount of grass which could conveniently be pulled out using the thumb and forefinger. The weight of the grass collected in this way ranged from 150-600g.

The total grass sample was treated by a modification of the method described by Smeal and Hendy[9]. Samples were weighed and soaked in 2 gallons of lukewarm tap water to which a small quantity of a non-ionic detergent ("Lissapol NDB", I.C.I. Ltd., England) was added. This was left overnight and then the grass was removed in small handfuls which were squeezed and transferred to a second bucket in

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which the washing process was repeated. This sample was allowed to stand for a further 24 hours before the grass was taken out and dried in trays first at room temperature and then in an incubator until completely dried. The buckets were left standing for 6 hours to allow the larvae to settle and the supernatant was then siphoned off. After mixing, the sample was passed through a 20 mesh per inch sieve to remove all the grass and transferred to graduated cylinders. This process of sedimentation was repeated until the sediment was concentrated to a volume of 250 ml then, after thorough mixing, one fifth was processed in the extraction apparatus shown in the diagram (Plate 1). Water passes upwards through the sediment in the vertical tube before being collected in 2 x 2,000 ml graduated cylinders. The flow of water was adjusted to a continuous drip and at 10 minute intervals the sediment was agitated by releasing the clamps. The total procedure of filling each of the 2 cylinders took approximately one hour. These cylinders were left standing overnight before the supernatant was decanted. The sediment was repeatedly washed and concentrated until a total volume of 10 ml was obtained and 0.5 ml of this was then transferred to each of 2 glass counting chambers[10] for microscopical examination. Infective larvae were identified and counted and, when necessary, due to large numbers of larvae or excess sediment, the volume was increased. After counting the number of larvae was

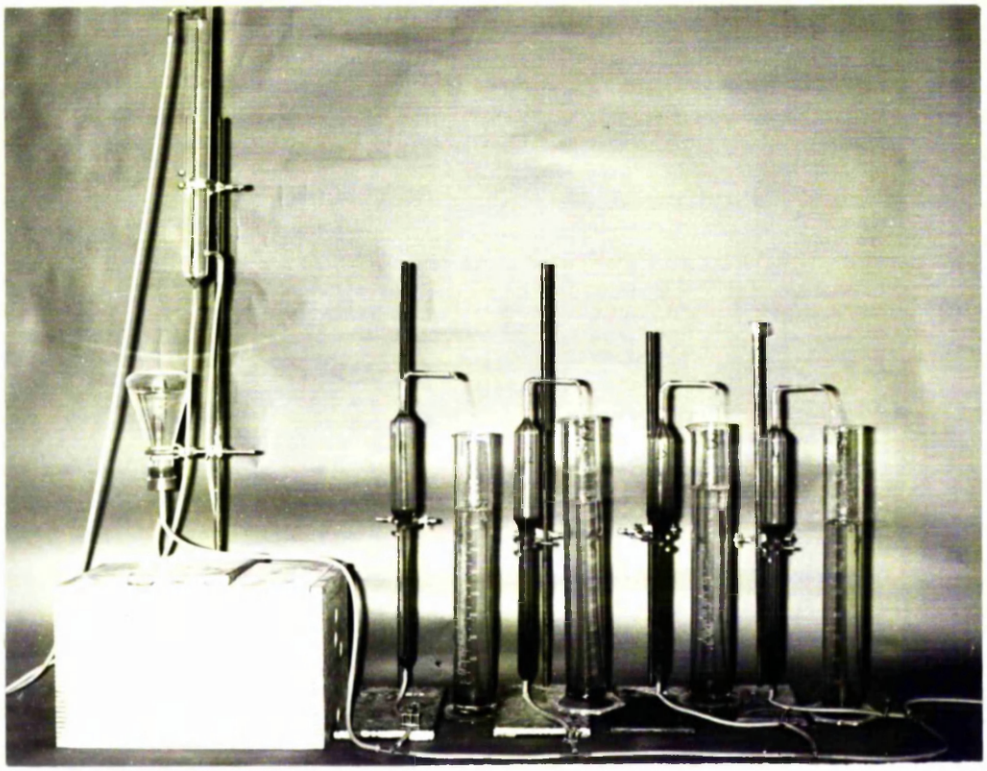

PLATE 1 Apparatus used by Smeal and Hendy to extract nematode larvae from grass samples.

multiplied by a dilution factor which expressed the result in larvae per kilogram of dried grass.

Since it was felt desirable to have some comparative data between this new method of Smeal's and the other currently in use in the laboratory, the remaining 200 ml of the 250 ml of sediment was passed through a double layer of milk-filters placed on top of a sieve on a Buchner funnel. These filters were then placed in a Baermann apparatus and left overnight before 10 ml was withdrawn and the larvae identified and counted as before. Total larval counts thus obtained were multiplied by a factor of 1.25 to compensate for the 50 ml which was processed by the extraction method and a final calculation was then made to express the results in larvae per kilogram of dried grass.

Identification of the different larval species was made according to the criteria outlined by Keith[11] and the Weybridge Manual of Veterinary Parasitological Laboratory Techniques[12].

Irradiation Procedure

O. ostertagi and H. contortus larvae were irradiated in a ^{60}Co gamma irradiation unit (Gamma Chamber Mark IV B. Nuclear Engineering Ltd., Southampton St., Reading, England). Before irradiation an approximate estimate of the number of larvae present was obtained by the procedure described above, except that in this case only 10 aliquots were counted. Larvae were then concentrated and pipetted into

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perspex test tubes held in a perspex rack (Plate 2) which was then placed in the central column of the ^{60}Co unit and lowered mechanically into the irradiation chamber. As the output of the machine was 2.5 Kr per minute, and the irradiation dose required was 60 Kr., larvae were exposed for exactly 24 minutes. After irradiation, the required doses were prepared for administration. The time between irradiation and dosing was never greater than 2 hours.

Irradiated D. viviparus larvae were obtained from Allen & Hanburys, Ware, Hertfordshire, in the form of the commercially available product 'Dictol'.

Necropsy Procedure

Details of slaughter

Twenty four hours before slaughter the animals were starved. They were then killed with a captive bolt pistol and immediately bled out and where appropriate the whole gastro-intestinal tract and/or the lungs removed.

Abomasum

A ligature was placed round the pylorus, and the abomasum together with the omasum separated from the small intestine, care being taken to prevent any loss of the abomasal contents. Immediately they were transferred to a graduated polyethylene bucket where the omasum was removed and discarded. The abomasum was opened along the greater curvature and samples of the contents taken to estimate the pH. The abomasum was then washed with lukewarm

PLATE 2 Irradiation chamber.

tap water and the volume of combined washings and contents adjusted to 4 litres in the case of calves, and 2 litres in sheep. After thorough mixing, 2 samples each of 200 ml were taken for estimation of the worm population and 10 ml of formalin added as preservative to each sample. The abomasal mucosa was scraped with a butcher's knife, finely chopped and then split in 200 g aliquots which were transferred into Kilner jars. These were subsequently filled with a mixture of pepsin and hydrochloric acid in the proportion recommended by Herlich[13] (10 g of 1:2,500 pepsin powder (BDH, Poole, Dorset, England), were dissolved in 600 ml of water and later 30 ml of concentrated hydrochloric acid added) and incubated at 42°C for 6 hours. The digests were then formalised, made up to 4 litres and 2 samples each of 200 ml were examined for parasitic larval stages.

Small intestine

The small intestines were separated from the mesenteric attachments and divided into 3 equal lengths. Each length was opened and washed under running water into a graduated bucket. The volume was made up to 4 litres and a single sample of 200 ml was taken and formalised as above.

Large intestine and caecum

The large bowel was opened and examined by the naked eye for the presence of worms or worm nodules.

In most instances no worms or lesions were detectable although occasionally a few Trichuris species were noticed in samples from grazing calves.

Lungs

Immediately after death, the lungs including the oesophagus and trachea were removed and photographed. Prior to dissecting the lungs the macroscopic pulmonary lymphoid nodules visible under the pleural surface were counted. These nodules are known to develop in calves following the administration of X-irradiated D. viviparus larvae and occasionally following infection with normal lungworm larvae[14,15,16]. Worms were recovered by the following procedure: The air passages were opened completely starting from the trachea and cutting down to the small bronchioles; visible worms were removed and put into a petri dish containing warm normal saline. At this stage samples for histopathology were taken from the tip of the right diaphragmatic lobe and from a medium sized bronchus. At the same time other portions of lung tissue were taken depending on the degree of emphysema, oedema or congestion. When the air passages were completely opened, the right and left lungs were divided and transferred to separate buckets containing approximately 1 gallon of warm saline. They were frequently squeezed gently between the fingers over a period of 3 hours, after which the lungs were again checked for the presence of any worms and discarded. The buckets were left standing for

one hour, then decanted and the sediment transferred to glass cylinders for final concentration. Worms were left overnight at 4°C to relax and become disentangled thus facilitating counting.

Worm counts

The preserved samples were completely mixed 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 1 litre with distilled water). After thoroughly stirring, a 10 ml "sawn-off" pipette was used to take 4 ml samples which were transferred to a petri dish, there they were decolourised by a few drops of a 5% solution of sodium thiosulphate and counted under a dissecting microscope (M5 Wild, Heerbrug, Switzerland). This procedure was effective as the worms retained the iodine stain and counting was therefore facilitated. Ten aliquots were examined, and the number of worms were multiplied by 100 in the case of calves abomasa or 50 for sheep abomasa, to find the total number of parasites.

It was not necessary to stain lungworms for counting as they were easily visible to the naked eye and could be picked out individually. After counting they were fixed in 10% formaldehyde.

Estimation of abomasal pH

The pH of the abomasal contents was determined within 30 minutes after slaughter, using a pH meter (pH 26, Radiometer Copenhagen, V.A. Howe & Co.Ltd., London, England).

Meteorological Data

The data referred to in this thesis was recorded by the Glasgow Weather Centre at Abbotsinch Airport situated approximately 5 miles from the Veterinary School.

Haemonchus contortus Measurement

Measurement of female H. contortus were made placing several worms on glass slides. Heated glycerin jelly was used as a mounting medium and a coverslip then applied. The worms were then projected on the screen of a Projectina microscope (Projectina Co.Ltd., Heerbrugg, Switzerland) using a 10X objective and 10X eyepiece. The visible image was traced on transparent paper, and the length of the worm image was measured in mm. using an opsometer.

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SECTION 1

IMMUNISATION OF MILK FED CALVES AGAINST *DICTYOCAULUS VIVIPARUS*.

INTRODUCTION

Parasitic bronchitis is an important disease of cattle caused by Dictyocaulus viviparus which has a worldwide distribution and there are reports of its occurrence in cattle, some dating from Greek and Roman times[1].

The life cycle of D. viviparus is direct and was first described by Daubney[2]. Since then there have been many studies on both the free-living and parasitic stages of the life cycle and the detailed life cycle is now considered to be as follows: Adult worms, which measure up to 8 cms in length, live in the main air passages in the lungs, i.e. the bronchi and bronchioles, and the fertilised female worms lay eggs which are coughed up and pass into the gastrointestinal tract where they hatch into first stage larvae (L_1). These larvae are voided in the faeces and under suitable conditions of moderate temperature and plentiful moisture develop through the L_2 stage to the infective L_3. In Britain this development may take as short an interval as one week in mid-summer but up to 4 weeks in spring and autumn; development during the winter is extremely slow[3,4]. The L_1 and L_2 stages are particularly labile; the L_3 stage is also susceptible to adverse conditions such as drought and freezing, and only a few survive for more than 3 months in England according to Michel and Rose[5]. However, in Scotland[6] and

Northern Ireland[7] it has been shown that larvae overwinter in sufficient numbers to produce an infection in calves grazing in the following spring. Larval stages are sluggish and do not migrate far from the faeces in which they develop unless disseminated by mechanical or human agencies[8] or via spores of the fungus Pilobolus[9].

When ingested by a susceptible host it is generally agreed that the larvae migrate to the lungs via the mesenteric lymph nodes, lymphatic blood vessels and blood and after 2 moults the adult stage is reached and L_1 appear in the faeces about 21 days after infection. The life cycle in the final host has been conveniently divided into 4 phases[10].

The first the penetration phase, is from day 1 to 7; during this early phase the larvae penetrate the intestinal wall, moult to the fourth larval stage in the lymph nodes, and then continue their migration to the lungs. At this time the animal does not present any clinical signs unless the infecting dose is massive.

The second, the prepatent phase, is from day 7 to 25; with as few as 200 lungworms there is a sudden increase in the respiratory rate at about 10 days after infection, coughing being quite frequent. At the end of the third week the symptoms lessen unless there are complications, although heavily infected animals (about 1,000 worms) may die around this time. At

post-mortem examination of early prepatent cases, the worms may not be seen with the naked eye due to their small size.

The third, the patent phase, is from day 25 to 55; this is characterised by a marked increase in the respiratory rate due to the presence of masses of adult worms blocking the air passages together with the aspiration of eggs and larvae into the lung parenchyma. Coughing becomes more frequent and emphysema and oedema contribute greatly to the clinical signs and fatalities which may occur. Food intake is reduced and consequently there is a decrease in body weight gain and in severe infections weight loss may occur. Larvae are present in the faeces.

The fourth, the post-patent phase, is from day 55 to 70; during this period animals slowly recover, the adult worms are eliminated and the respiratory rate gradually returns to normal and coughing becomes less frequent. As a result most animals begin to gain weight but some may remain permanently unthrifty due to persistent lung lesions.

Two features of the life cycle make the control of parasitic bronchitis by conventional means of pasture management and/or anthelmintic prophylaxis unlikely. These are:

a) the short period of 5-7 days required in mid-summer for the L_1 to reach the infective L_3 stage.

b) the comparatively small numbers of lungworms (in excess of 200) which are sufficient to cause respiratory distress and impair productivity.

Despite this, however, the disease has long been recognised as being mainly confined to young cattle, particularly of the dairy breed, where the normal husbandry practice is to graze these separately from older stock. Thus, field observations[11,12,13] which suggest that calves, once exposed to infection with D. viviparus, develop a high degree of acquired immunity, has been confirmed under experimental conditions by several workers. Thus, Porter and Cauthen[14] found that following a primary infection with lungworm, larvae were present in the faeces of calves for a longer time than in the faeces of older cattle. On reinfection the young cattle were partially resistant with only a few larvae appearing in the faeces whereas the older cattle were completely resistant with no larvae in the faeces. Further studies by Michel[15] showed that when previously infected calves were given an experimental challenge infection of lungworm, L_1 did not subsequently appear in the faeces although a severe clinical reaction occurred 1-2 weeks after challenge. Rubin and Lucker[16] also demonstrated a strong resistance to reinfection with lungworm and noticed that when the challenge was extremely high a severe clinical reaction occurred. If, however, the calves were subjected to repeated reinfection

they became highly immune, the faeces remaining free of L_1 and adverse clinical reactions did not occur. Weber and Lucker[17] found that a primary infection with normal larvae protected calves against the effects of a subsequent challenge with 25,000 L_3 in terms of clinical signs, although a few adult lungworms became established and L_1 appeared in the faeces.

More positive evidence for an experimentally acquired immunity induced by normal D. viviparus larvae was reported by Jarrett, Jennings, McIntyre, Mulligan, Thomas and Urquhart[18] who infected each of 10 calves with doses of 2,500, 4,500 and 13,000 L_3 with a 5 month interval between each dose. On faecal examination, all 10 calves excreted larvae after the first infection, only 4 after the second and merely two after the final dose. At post-mortem examination 32 days after the last infection only a few adults and immatures were recovered from the lungs of 3 calves, the other 7 pairs of lungs being negative. In another experiment, the same workers infected 5 calves with 25 doses of 300 D. viviparus L_3 every 2 or 3 days for 2 months; 5 months later 4 of the calves were challenged with 15,000 L_3 and the fifth with 90,000 L_3 and all were slaughtered 30 days later. During the trickle infection 5 calves excreted L_1 in their faeces but the larval output had fallen to zero by the time of the final infection. After challenge they showed consistently negative results on faecal examination and the mean worm recovery for the 4 calves challenged with 15,000 larvae was 22, while only 32 immature worms were

recovered from the lungs of the fifth calf given 90,000 L_3.

Subsequently, Michel[19] demonstrated, on the basis of worm recoveries, that calves given a primary infection of between 3,200 and 3,500 L_3 were resistant to a challenge of 35,000-40,000 L_3 given at variable intervals after the primary infection. This resistance became apparent as early as 11 days after the primary infection, appeared to increase to a maximum between 2-3 months post-infection and then gradually declined.

From all these experimental studies it is apparent that previous exposure to normal D. viviparus L_3 confers a substantial degree of resistance to reinfection.

The first attempt to experimentally induce an immunity to parasitic bronchitis was made by Jarrett, Jennings, McIntyre, Mulligan and Urquhart[20] who used hyperimmune sera produced by giving repeated experimental infections of D. viviparus to cattle which had recovered from natural infection with lungworm. The globulin fraction from these sera, when administered intra-peritoneally to susceptible calves, conferred a significant degree of protection against challenge with 4,000 L_3 2-5 days later. Rubin and Weber[21] failed to transfer a significant degree of immunity with hyperimmune serum, and 3 of the 5 animals given the serum plus the controls died following challenge with 50,000 D. viviparus L_3; however, this challenge dose was exceedingly high and may account for the discrepancy between these results and

those of Jarrett and his co-workers[20].

Serological studies in calves infected with lungworm[22] showed that complement fixing antibodies appeared approximately 2 weeks after a primary infection, reaching a peak a few days later and persisting for about 2 months. When adult worms were incubated in this serum they showed precipitates around their orifices which were maximal in sera collected 4 weeks after infection[23]. However, both Jarrett et al.,[18] and Michel and Cornwell[24] found that there was little correlation between the titre of complement fixing antibodies and resistance to lungworm by the host. Later, Cornwell and Michel[25] found that when the intake of larvae was continuous antibody levels remained steady, but if the larval intake was reduced there was a proportionate drop in the titre. When the dose of larvae was then increased there was a typical secondary response with an immediate rise in antibody level.

Following the partially successful attempts to passively immunise cattle with hyperimmune serum Jarrett, Jennings, McIntyre, Mulligan and Urquhart[26] attempted vaccination using an adjuvanted whole lungworm vaccine, but the results obtained were equivocal. Since neither passive immunisation with sera nor active immunisation with dead antigen with or without adjuvant had proved a practical proposition for immunisation against D. viviparus, these workers then turned their attention to the use of an attenuated live vaccine.

The use of irradiation as a means of attenuating nematode larvae was first shown by Tyzzer and Honeij[27] in 1916, using radium-irradiated Trichinella spiralis larvae; these attenuated larvae, encysted in muscle strips, were non-infective when subsequently fed to mice. Schwartz[28] and Semrad[29] repeated these experiments in rats and found that attenuated T. spiralis larvae either failed to develop or developed into sterile adults. Later, Levin and Evans[30] and Gould, Gomberg, Bethell, Villella and Hertz[31] demonstrated that rats fed gamma-irradiated T. spiralis larvae were partially immune to challenge with normal larvae.

Following a preliminary titration experiment using X-irradiation Jarrett, Jennings, McIntyre, Mulligan and Urquhart[32] found that a dose of 40 Kr gave the most satisfactory attenuation of D. viviparus larvae; the majority of the attenuated larvae reached the mesenteric lymph nodes but only a few female worms survived to reach the lungs (the male parasite is more susceptible to the effects of ionising radiation) and clinical respiratory signs did not occur. Calves given a double immunising dose of X-irradiated larvae were immune to subsequent challenge with 10,000 normal D. viviparus L_3[33]. Later, Jarrett, Jennings, Martin, McIntyre, Mulligan, Sharp and Urquhart[34] tested the irradiated larval vaccine in a small scale field trial; in this trial 5 infected calves with patent lungworm infections were grazed for 40 days on a 3-acre pasture. These 'seeder' calves were then removed and 10 more

'seeder' calves, together with 15 vaccinated and 12 uninfected control calves were permitted to graze the paddock. The vaccinated animals received 1,000 X-irradiated infective larvae 50 days prior to grazing the pasture. The pasture challenge to the vaccinated animals (originating from the 'seeder' calves) was extremely high and estimated at 1,300 D. viviparus L_3 per square foot; this was augmented via the control calves which had become infected during the experiment. The vaccinated animals proved to be highly immune as assessed by the clinical and pathological changes, mortality, morbidity, faecal larval counts and worm burdens.

A large scale field trial involving 1,088 calves on 40 commercial farms with a previous history of parasitic bronchitis was also undertaken by Jarrett, Jennings, McIntyre, Mulligan and Urquhart[35]. Half of the calves on each farm were vaccinated, the remaining half serving as controls. Parasitic bronchitis occurred on 6 of the farms and the effectiveness of the vaccine was demonstrated by the fact that on these farms only 6% of the vaccinates were affected, compared with 62% of the controls.

In view of this encouraging result, another experiment was conducted by Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart[36] using 50 calves aged 10 weeks and divided into 5 groups. The calves of Groups 1, 2 and 3

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were each immunised with 1,000 X-irradiated larvae followed 6 weeks later by a second dose of 4,000; 2,000 and 1,000 L_3 X-irradiated larvae respectively. The fourth group received only the first dose, and the animals in Group 5 served as controls. On day 93 all five groups were challenged with 10,000 normal larvae with the exception of 5 controls given only 5,000 L_3. There were mild and transient increases in the respiratory rates of the double vaccinated calves after each immunising dose, but during and after challenge these values were declining. Group 4, however, which received only the first vaccine, showed a marked increase after challenge, as did all the controls. The five groups of calves were killed on day 125. There was very little difference in the numbers of worms recovered from the control animals and those in Group 4 (approximately 1,000 and 800 respectively) although in the latter the lesions were less severe. These calves which were double vaccinated before challenge presented almost no lesions at necropsy and no adult worms were recovered. There were also marked differences in weight gains, the percentage weight gain being approximately 18% for the double vaccine groups, 7.6% for the single vaccine calves and 5% for the controls.

Following the successful immunity produced by the double vaccination, Jarrett, Jennings, McIntyre, Mulligan and Sharp[37], double vaccinated 5 calves, and turned them and 5 controls on to pasture contaminated with lungworm larvae. The protection afforded by the vaccine

was demonstrated by the lower respiratory rates, better weight gains, lack of mortality and absence of larvae in the faeces of the vaccinated calves. One control died of severe parasitic bronchitis and at post-mortem examination only a few immature worms were found in the lungs of the vaccinates, whereas the control group had a mean of over 400 parasites.

Following these studies an X-irradiated lungworm vaccine was produced commercially as Dictol by Allen & Hanburys Ltd., Ware, England, in 1959. The vaccine is recommended for use in weaned calves aged at least 8 weeks and reared free from infection of D. viviparus. Two doses are given at an interval of 4 weeks and it is recommended that a period of at least 2 weeks elapses between the final vaccination and exposure to infection at pasture. Following 2 years of commercial use of this vaccine, Nelson, Jones and Peacock[38] reported that from 8,000 farms where vaccination was carried out, only 28 outbreaks of parasitic bronchitis had been reported, and in 7 of these only a single animal was involved. In another 6 of the farms complicating respiratory disease was present. Over this period of 2 years the prevalence of husk in farms using the vaccine was reduced to less than 0.5%. A decade later Poynter, Peacock and Menear[39] reported that the prevalence of infection in vaccinated farms was 0.35%. It is interesting that in Switzerland where vaccination is compulsory on farms where parasitic bronchitis is diagnosed the prevalence of the disease has fallen over

a period of 3 years (1971-1973) the percentage of clinical cases dropping from 57% to 15%; the number of calves excreting larvae has reduced from 62% to 37%. (Eckert, personal communication).

Since the attenuation of D. viviparus larvae by X-irradiation was first reported, Cornwell and Jones[40] have successfully attenuated lungworm larvae using the cytotoxic agent triethylene melanine (TEM) at a concentration of 0.7% for one hour at 26°C. Two doses of 1,000 D. viviparus L_3 treated in this way provided an excellent immunity against challenge as measured by faecal larval excretion, respiratory rate and weight gains. A field trial of this vaccine[41] gave similar results to those obtained with X-irradiated larval vaccine. To date this vaccine has not been used on a commercial basis.

Although protection conferred by the X-irradiated larval vaccine has proved outstandingly successful under field conditions, some problems may arise following its use and these are being studied by workers in England. Thus, Cornwell[42] showed that previously vaccinated animals can develop patent infections after being subjected to field challenge and so maintain a reservoir of infection on the farm. In another experiment designed to assess the significance of these infections, Cornwell and Berry[43] showed that vaccinated calves could effectively act as carriers in the same way as the animals immunised

by exposure to normal larvae on the pasture, although their faecal larval output might be fairly low. In these experiments vaccinated calves previously grazed for 2-4 weeks on an infected farm were transferred to clean pasture and grazed alongside susceptible calves. The susceptible calves subsequently became heavily infected with D. viviparus, apparently originating from the vaccinated animals. As a result of this experiment it was recommended that vaccinated and unvaccinated calves be grazed separately and for efficient control of the parasite at farm level it was suggested that all calves should be vaccinated. This last point was clearly demonstrated by Downey[44,45] following studies on the efficacy of the vaccine under Irish conditions. In his trial Dictol vaccinated and non-vaccinated calves were grazed together on pasture known to be heavily contaminated with lungworm larvae. A massive build-up in the numbers of larvae on the pasture occurred via the unvaccinated animals and resulted in the death of 60-80% of these and 18% of the vaccinates.

Results confirming the efficacy of Dictol have now been reported from many countries. For example, in France by Pierre, Euzeby, Malher and Jeannin[46]; in the U.S.A. by Englebrecht[47]; from Sweden by Olson[48]; from Holland by Van Eck, Kruize, Paul, Reinders and Wilson[49]; from Belgium by Verycruysse, van Vliet and Kruize[50]; in Germany Enigk and Duwel[51,52] have reported

favourably on the efficacy of Dictol and more recently in Switzerland Eckert[53] has confirmed these observations.

The duration of the immunity acquired by calves following Dictol vaccination was questioned by Michel, McKenzie, Bracewell, Cornwell, Elliot, Hebert, Holman and Sinclair[54]. These workers vaccinated calves with Dictol or normal larvae and subsequently challenged these calves together with controls at 3, 6, 12, 18 and 27 months after immunisation. The challenge dose ranged from 7,500 to 30,000 normal larvae. At 3 months after the first vaccination the immunity obtained with Dictol, as measured by a comparative number of lungworms present at post-mortem, was 23%. At 6 months it was 44%; at 12 months, 41%, and at 18 months 39%. In contrast, immunity in the calves given normal larvae was 99%, 82%, 81% and 83% at 3, 6, 12 and 18 months respectively. This inexplicable result certainly differs from the results of previous studies with Dictol and also with the results obtained by Pirie, Doyle, McIntyre and Armour[55]; in the latter studies groups of 5 calves each, were double vaccinated with Dictol at an interval of one month and challenged 30 days later, together with control calves. At post-mortem the reduction in the lungworm burden of the vaccinate calves was 97% less than those present in the controls. If the challenge dose was delayed until 4 months after immunisation the reduction in the worm burden obtained as compared with controls fell slightly to 89%.

Until now the manufacturers of the commercial vaccine have recommended that only calves aged 8 weeks and over and reared free from lungworm infection should be vaccinated. In practice this has largely restricted the use of the vaccine to autumn and winter born calves in dairy herds. At the present time just under one million calves are vaccinated annually in the U.K. (Poynter, personal communication). Although this is a sizeable achievement in the prevention of parasitic bronchitis, beef calves suckling at grass and late spring born dairy calves remain relatively unprotected. Lungworm vaccination could also be applied to the latter groups if it could be demonstrated that 1) calves aged 3-4 weeks responded to immunisation in a competent fashion, 2) that the ingestion of large quantities of milk over the period of vaccination does not interfere with the infectivity of the irradiated larvae and subsequent immunogenicity, and 3) that small numbers of D. viviparus larvae ingested before or during vaccination might interfere with the full development of immunity.

The object of the experiment described in this section of the thesis was the elucidation of the first two questions posted above; if a satisfactory answer is obtained to these questions it is hoped to subsequently investigate the third point in the future.

EXPERIMENT 1

Object

The object of this experiment was to compare the immunity produced following Dictol vaccination of milk fed calves at 3 and 7 weeks old with that in weaned calves vaccinated at 8 and 12 weeks old.

Experimental Design

During the month of February 1974, a group of 5 calves were vaccinated with Dictol when 3 and 7 weeks old together with another group of 5 aged 8 and 12 weeks. Both vaccinated groups were challenged with normal *D. viviparus* infective larvae L_3 at a rate of 66 L_3/kg body weight 4 weeks after the second vaccination together with 2 control groups of 5 calves each. The design of the experiment is summarised in Table 1.

Observations

Prior to the first vaccination and throughout the experimental period the animals were clinically examined each day. At weekly intervals the calves were weighed and faeces collected from the rectum for *D. viviparus* larval examination.

At necropsy the lungs were removed and the number of lymphoid nodules on the surface counted; thereafter the trachea, bronchi and bronchioles were incised, the lungworms removed and enumerated.

TABLE 1

Experimental Design

Group Number	1	2		3	4
Number of Calves	5	5		5	5
Age			Age		
3 weeks	V	-	8 weeks	V	-
7 "	V	-	12 "	V	-
11 "	C	C	16 "	C	C
15 "	K	K	20 "	K	K

V = Vaccination

C = Challenge

K = Killed.

Results

One control calf from Group 2, aged 11 weeks died of extraneous causes before challenge, therefore this group was reduced to 4 animals.

Clinical Observations

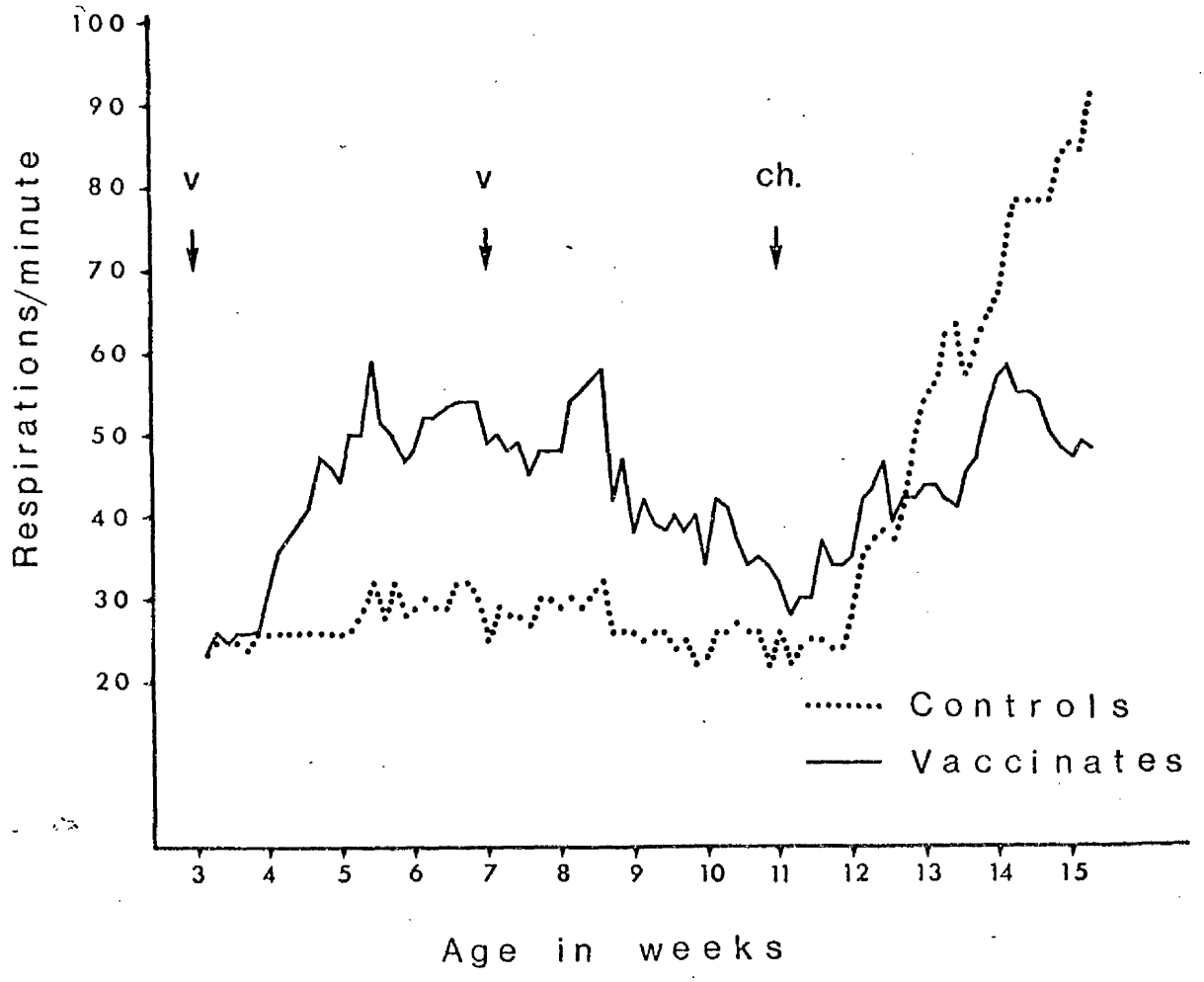
The vaccinated calves maintained good body condition and normal appetite throughout the experiment. In contrast, the controls showed an increasing degree of inappetence about 3 weeks after challenge and rapidly lost condition.

Respiratory rates

The mean respiratory rates of the younger calves, that is Groups 1 and 2 are shown in Fig. 1 and the individual values in Appendix A, Table 1. In the vaccinated calves (Group 1) the mean respiratory rate of 25/min increased sharply one week after vaccination reaching 59/min by the third week; thereafter the mean rate dropped slightly only to rise again to 58/min by 2 weeks after the second vaccination. Over the next 2 weeks the respiratory rates returned almost to the pre-vaccination level at 32/min. Following challenge the rate had increased by 3 weeks to a similar level (58/min) to that recorded after vaccination and then decreased to a mean of 48/min by slaughter one week later.

Figure 1 Mean respiratory rates of 5 pail-fed milk calves vaccinated with Dictol and 3 and 7 weeks-old and challenged 4 weeks later with D. viviparus (66 L_3/Kg) together with 5 controls.

V = Vaccinated Ch = Challenge

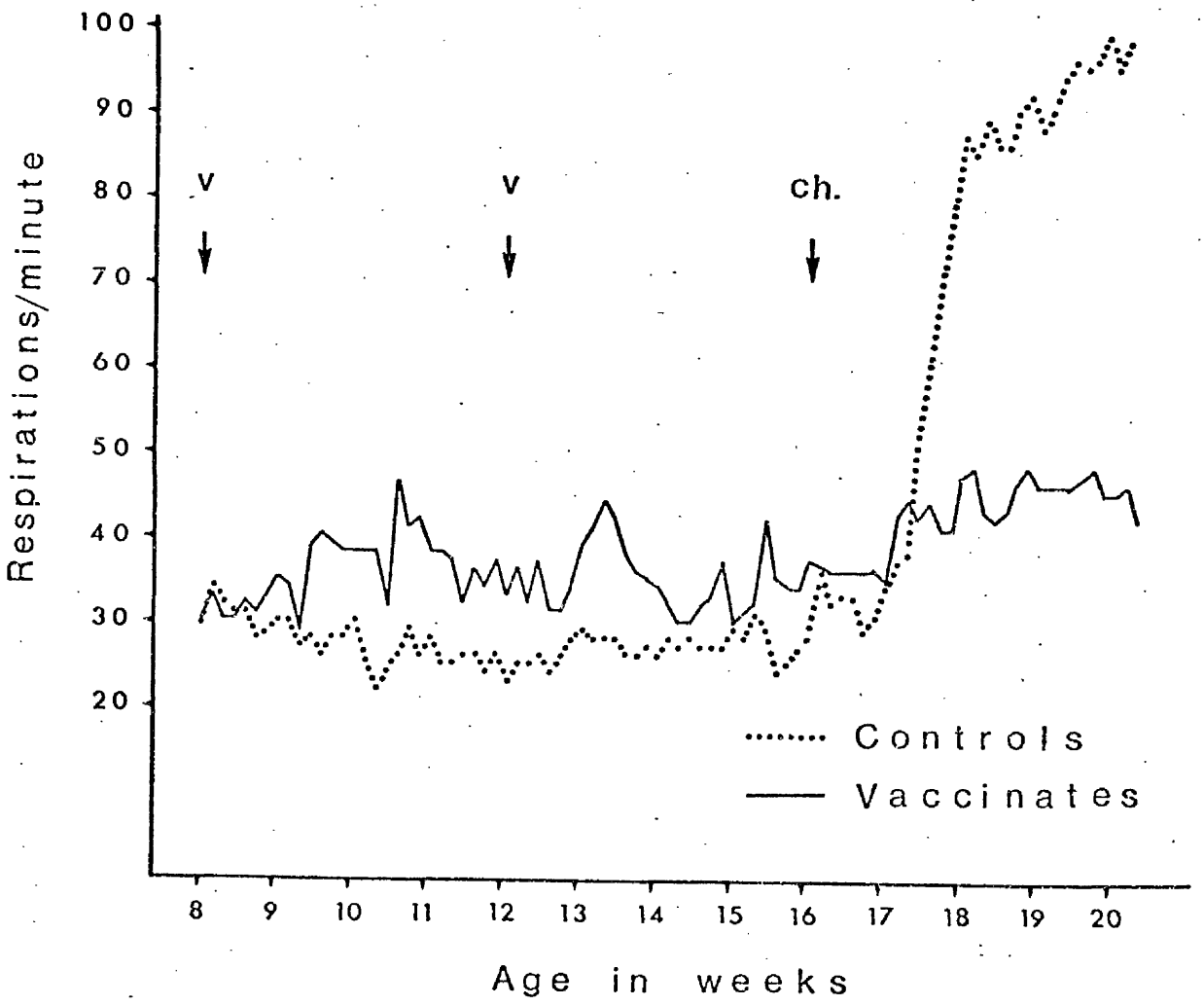


The control animals (Group 2) had a normal respiration rate until challenge. One week after challenge they showed a steady increase in their respiratory rates and by the end of the experiment 3 weeks later the mean rate was extremely high (91/min) and the animals were dyspnoeic.

The respiratory rates from the older calves, Groups 3 and 4, are shown in Fig. 2 and individual values in Appendix A, Table 2. The vaccinated calves (Group 3) showed a similar pattern as the Group 1 calves although the mean values were in general lower; following the first vaccination the mean respiratory rates rose to 46/min during the third week but decreased to pre-vaccination level by the time of the second vaccination. The latter was followed by another increase to a mean of 44/min after 2 weeks but at the time of challenge the mean rate was again below 40/min. After challenge the vaccinated group increased their respiratory rates during the second week and remained fairly constant around 45/min thereafter. The control group of calves (Group 4) showed normal respiratory rates until one week after challenge when it increased sharply and during the last 2 weeks the mean respiratory rate was around 87-98/min.

Apart from the increases in the respiratory rates the vaccinated group showed no clinical signs other than an occasional cough throughout the experimental

Figure 2 Mean respiratory rates of 5 weaned calves vaccinated with Dictol at 8 and 12 weeks-old and challenged 4 weeks later with D. viviparus (66 L_3/Kg) together with controls.
V = Vaccinated Ch = Challenge.



period. In the control groups, however, the increased respirations were of the harsh bronchial type (ronchi). Frequent coughing was observed together with distressed breathing, this becoming more marked as the experiment progressed and as a result one control calf in the older group (Group 4) died 14 days after challenge. At no time were rales noted.

Body Weights

The mean body weights of the calves from Groups 1 and 2 are shown in Fig. 3 and those of Groups 3 and 4 in Fig. 4. Individual values are shown in Appendix A, Table 3. All body weights increased normally in the vaccinates until the end of the experiment, but the control calves showed weight loss 3 weeks after challenge and this continued until sacrifice one week later.

Parasitological Data

Faecal examinations

The faeces of all vaccinated calves (Groups 1 and 3) were consistently negative for lungworm larvae throughout the experiment, i.e. after both vaccination and challenge. The faeces of the non-vaccinated calves (Groups 2 and 4) became positive for lungworm larvae during the 4th week after challenge reaching a mean maximum of 1,012 L_1/g in Group 2 and 400 L_1/g in Group 4. The larval counts per gram are shown in Appendix A, Table 4.

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Figure 3 Mean body weights of 5 pail-fed milk calves vaccinated with Dictol at 3 and 7 weeks-old and challenged 4 weeks later with D. viviparus (66 L_3/Kg) together with 5 controls

V = Vaccinated Ch = Challenge.

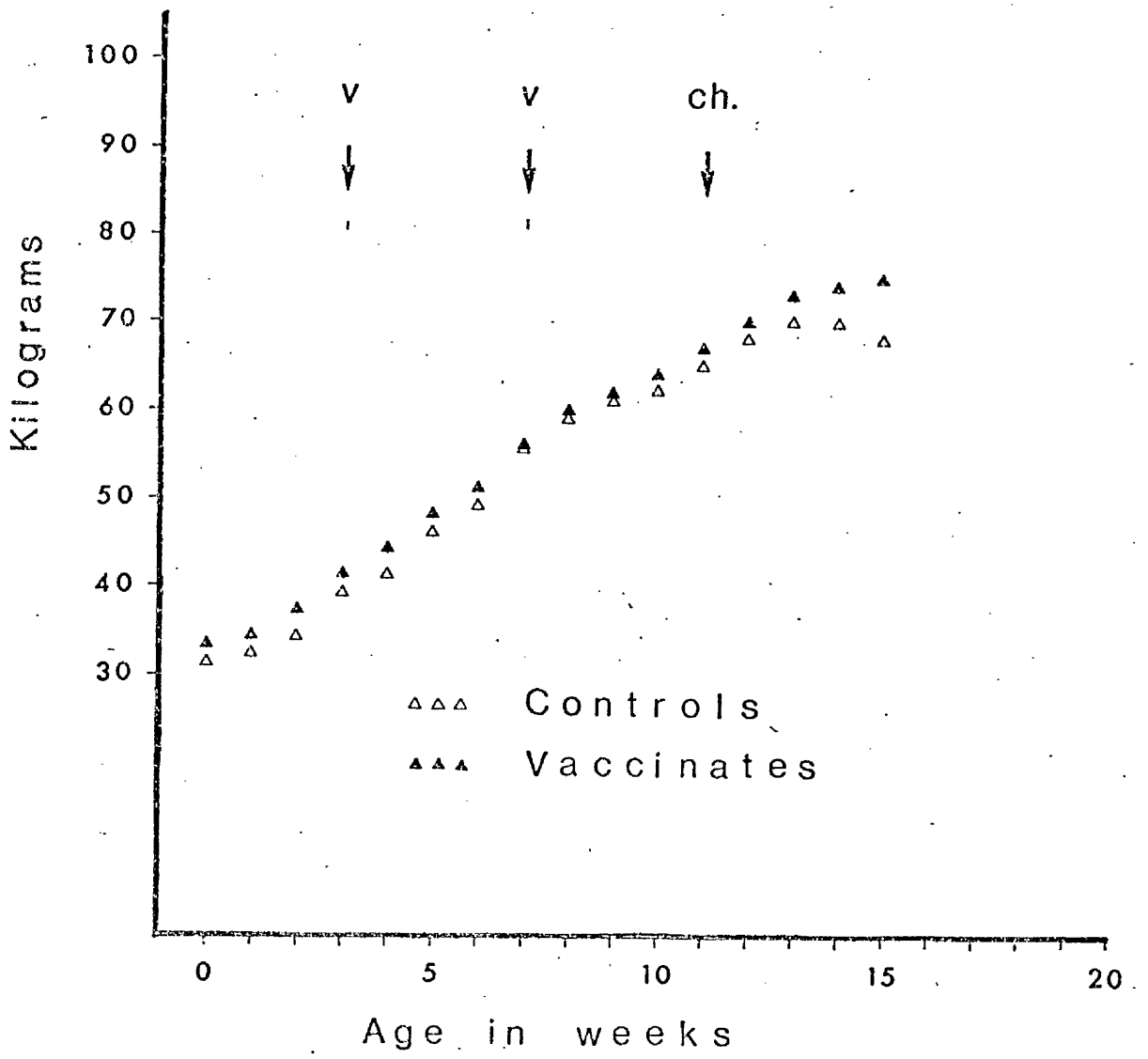
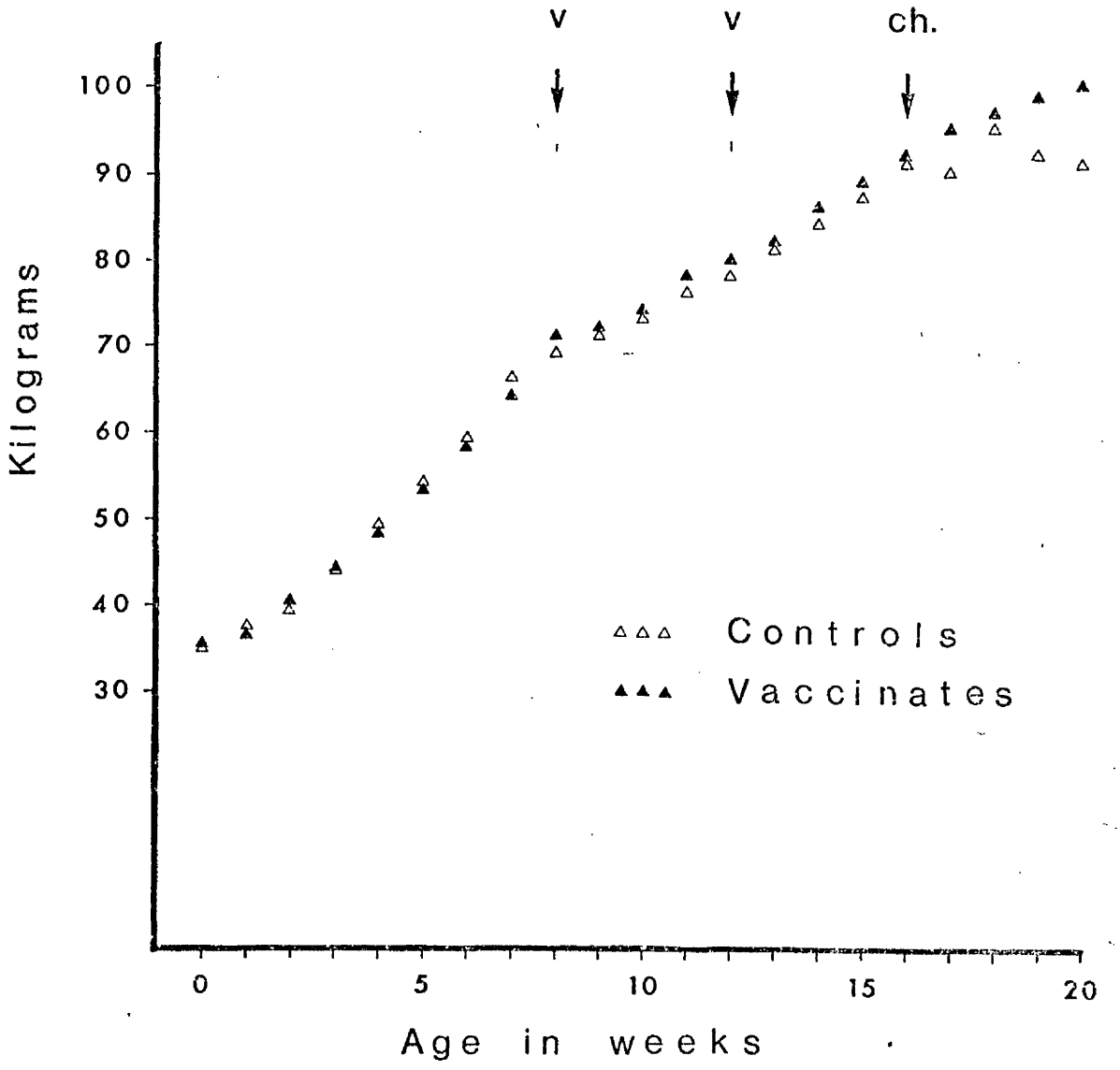


Figure 4 Mean body weights of 5 weaned calves vaccinated with Dictol at 8 and 12 weeks-old and challenged 4 weeks later with D. viviparus (66 L_3/Kg) together with controls

V = Vaccinated Ch = Challenge.



Serological Results

As shown in Fig. 5, each point of which is calculated as the mean logarithm to the base of 2 of the titres, the mean antibody levels measured by the passive haemagglutination technique in Group 1 remained fairly constant until 3 weeks after the first vaccination when there was a marked rise in the mean values over the next 3 weeks and then a steep drop until the animals were challenged with D. viviparus L_3. From then onwards a gradual increase again occurred which surpassed the previous titres and reached a mean value of 8.2 ± 0.4. In the control calves (Group 2) the titres began to increase on day 14 after challenge and reached a mean of 6.5 ± 0.6 at the termination of the experiment.

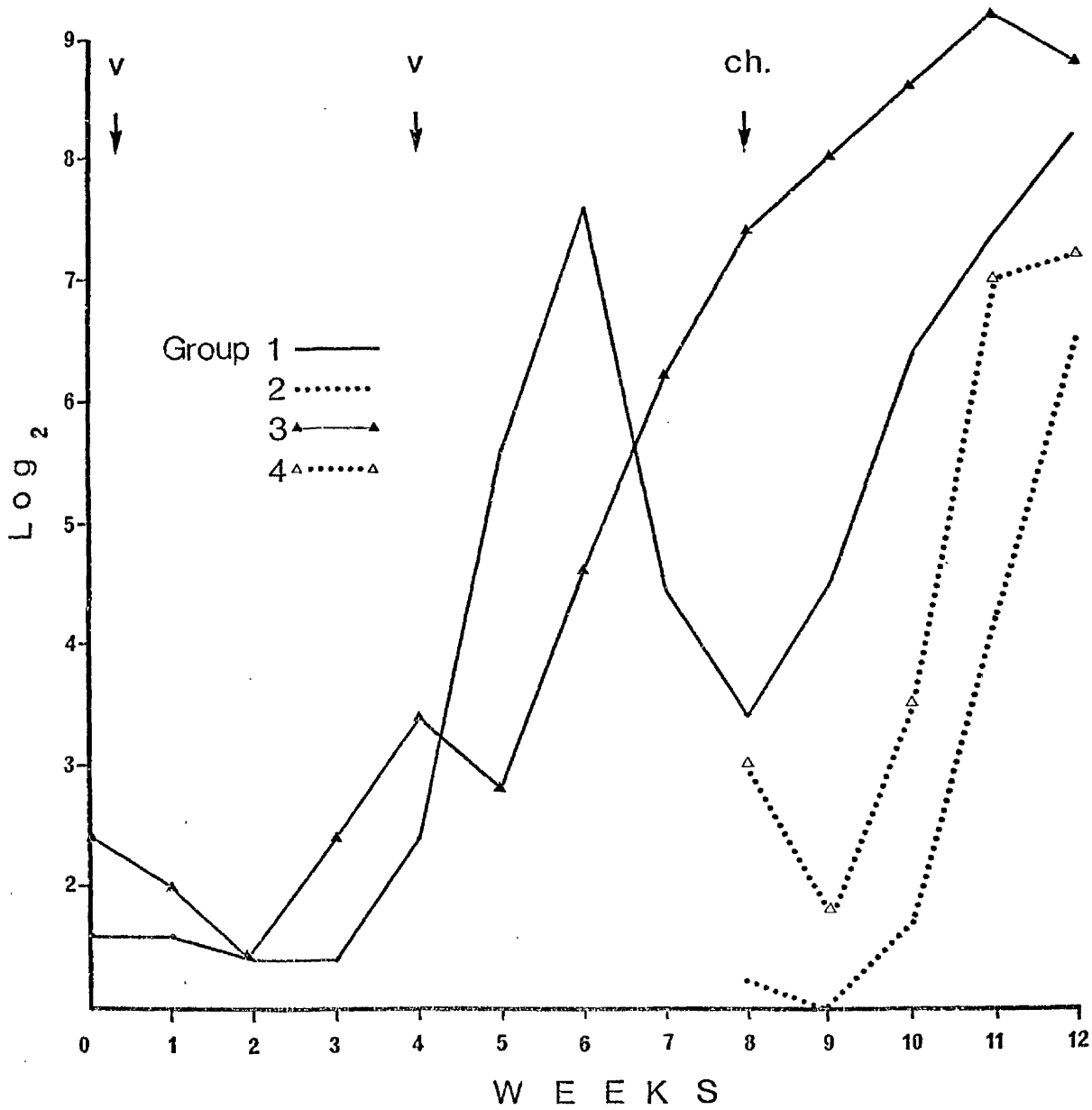
In the older animals of Group 3 there was a slight fluctuation of antibody titres up to the 5th week after primary vaccination and then a steady increase to a mean value of 7.4 ± 0.7. Following challenge there was no marked anamnestic response. The control Group 4 showed the same pattern of response as in the other control group (Group 2). Individual values are presented in Appendix A , Table 5.

Post-mortem Data

Lung lymphoid nodules

Numerous lymphoid nodules were present on the surface of the lungs from the vaccinated calves; the mean counts per pair of lungs being 141 and 173 in Groups 1 and 3

Figure 5 Mean haemagglutination titres of 4 groups of calves. Groups 1 and 3 were vaccinated with Dictol at 3 and 7 and 8 and 12 weeks respectively. These groups and their respective controls (Groups 2 and 4) were challenged 4 weeks after the second vaccination.



respectively; in contrast the control animals (Groups 2 and 4) had only a mean of 2 and 4 respectively. The number of nodules of each animal are shown in Table 2.

The visceral lung surface from a vaccinated calf with a total of 140 nodules is shown in Plate 1 (3 nodules indicated) and that from a control calf with a total of 3 nodules in Plate 2 (1 nodule indicated).

Worm recoveries

The mean number of D. viviparus recovered from the calves in different groups are shown in Table 2. A mean of only 28 and 30 lungworms were obtained from the vaccinated Groups 1 and 3, while the control groups had a mean of 861 and 2,789 D. viviparus respectively. The dissected lung of a vaccinated calf (199) is shown in Plate 3 and that from a control calf (232) in Plate 4.

TABLE 2

A comparison of the resistance to experimental challenge of two groups of calves double vaccinated with Dictol; in one group (1) vaccination commenced at 3 weeks old and in the other at the recommended age of 8 weeks (3).

Group	Calf Number	Challenge Dose L_3	D. viviparus established at p.m. on day 30	% Reduction in worm burden compared with controls	Lymphoid nodules
1	192	4,830	22	97.4	128
	194	3,750	5	99.4	163
	195	5,070	28	96.7	146
	201	3,420	14	98.4	140
	203	5,010	71	91.8	126
Mean		4,416	28	96.7	141
S.E.		345	11	1.3	6.7
2.	190	4,200	947		1
	191	3,930	442		3
	200	4,110	1,278		-
	236	5,110	777		4
Mean		4,335	861		2
S.E.		264	174		0.9
3	193	6,000	4	99.8	166
	197	7,020	-	100.0	173
	199	6,570	48	98.3	156
	202	5,850	26	99.0	198
	237	5,160	72	97.4	Impossible to count due to emphysema
Mean		6,120	30	98.9	173
S.E.		318	13.6	0.5	8.9
4	205	7,140	2,993		5
	232	5,220	2,503		7
	233	7,350	2,696		-
	234	5,850	2,963		4
	235	4,410	Died 14 days after challenge		-
Mean		5,994	2,789		4
S.E.		560	116		1.5

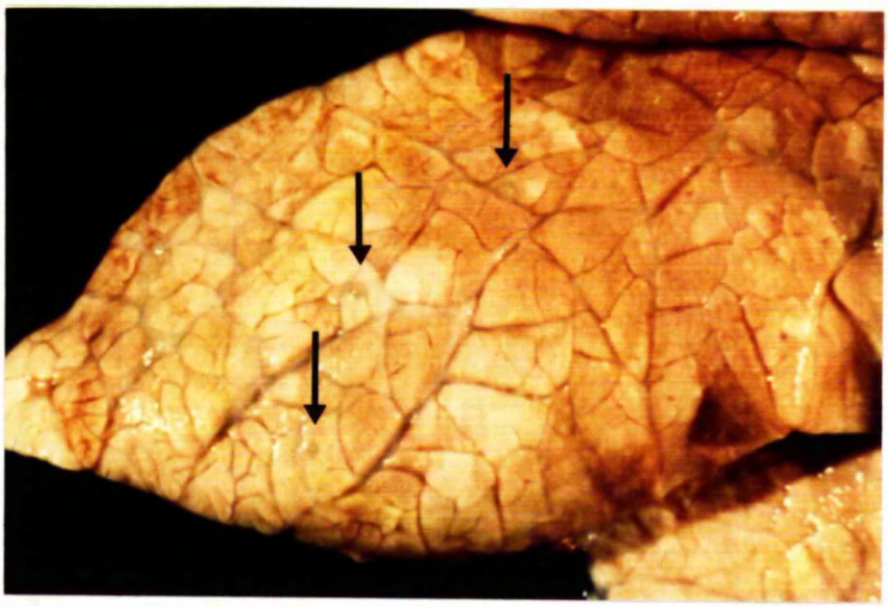


Plate 1 Visceral lung surface from a Dictol-vaccinated calf (201) showing 3 lymphoid nodules (indicated by arrows).

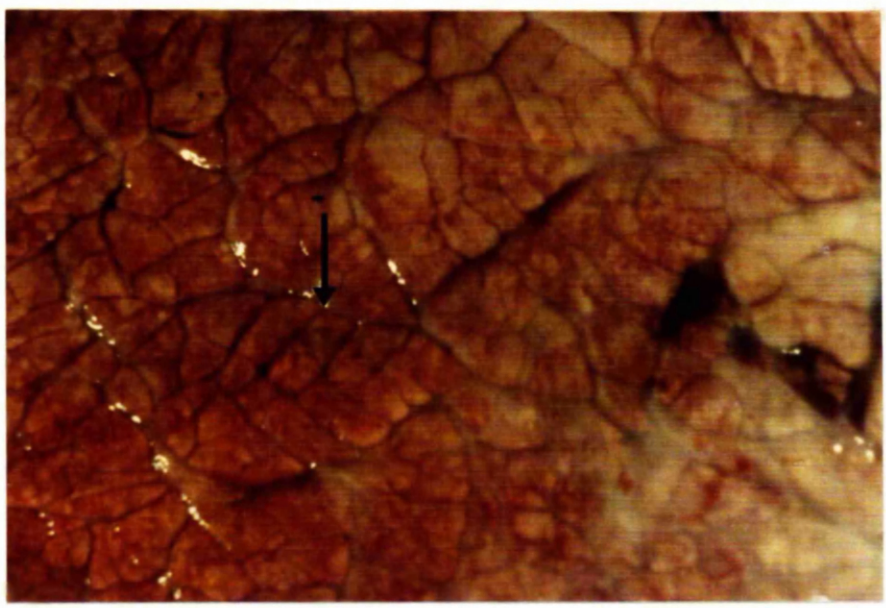


Plate 2 Visceral lung surface from a control calf (191) showing one lymphoid nodule (indicated by an arrow).

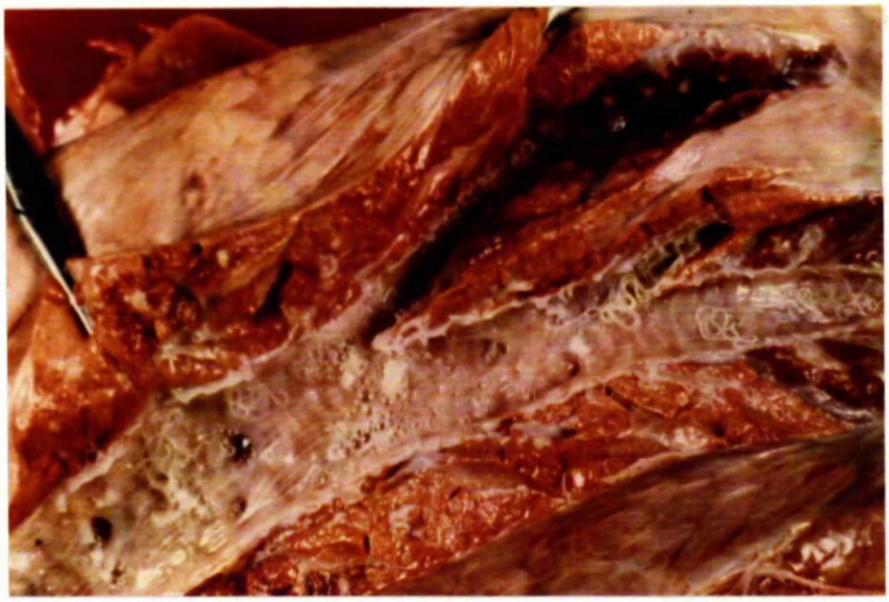


Plate 3 Dissected lung from a Dictol-vaccinated calf (199) 4 weeks after challenge with D. viviparus L_3 showing very few lungworms.

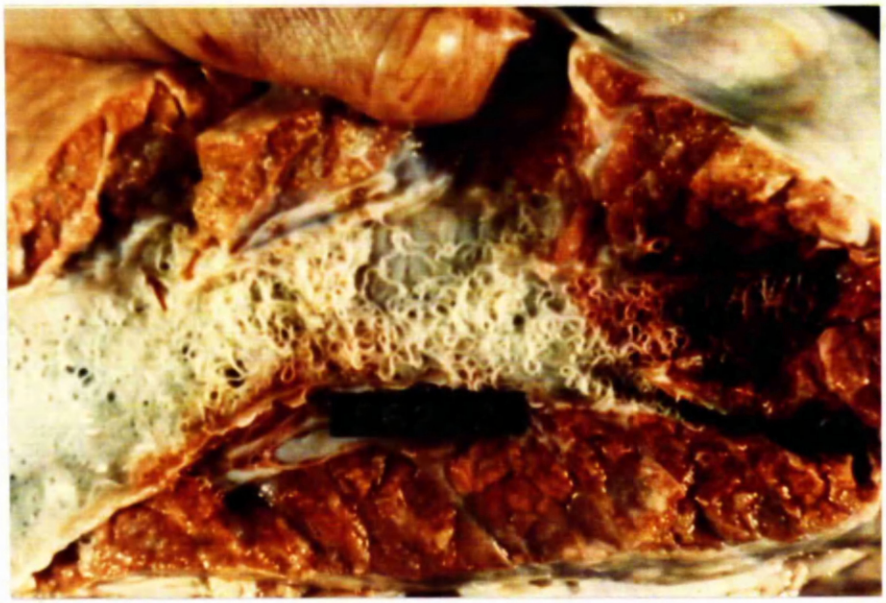


Plate 4 Dissected lung from a control calf (232) 4 weeks after challenge with D. viviparus L_3 showing masses of lungworms.

EXPERIMENT 2

Object

The object of this experiment was to study the immunity produced by vaccination of suckling calves at 3 and 7 weeks old with Dictol.

Experimental Design

In August 1974, 12 calves aged 1 to 3 days old were randomised into 3 groups of 4 and each group allocated to suckle a foster cow. Two calves from each cow were vaccinated with Dictol at 3 and 7 weeks of age and challenged experimentally at 11 weeks together with the remaining 6 control calves. The allocation of calves to vaccinated and control groups was based on body weights and zinc sulphate turbidity values, 2 calves from each foster cow being placed in each group. The design of the experiment is shown in Table 3, and the body weights and globulin levels of the calves are shown in Table 4.

Observations

Before vaccination and throughout the experiment the animals were subjected to daily clinical examinations. At weekly intervals the calves were weighed and faeces collected from the rectum for *D. viviparus* larval counts.

Results

Clinical Observations

Prior to and following vaccination, all animals had a normal appetite and were in good body condition. After

TABLE 3

Design of Experiment 2

Group	1	2
Number of calves	6	6
Age		
3 weeks	V	-
7 "	V	-
11 "	C	C
15 "	K	K

V = Vaccination

C = Challenge

K = Killed.

TABLE 4

Immunoglobulin levels measured by the turbidity test at one week of age and body weight at 3 weeks of age.

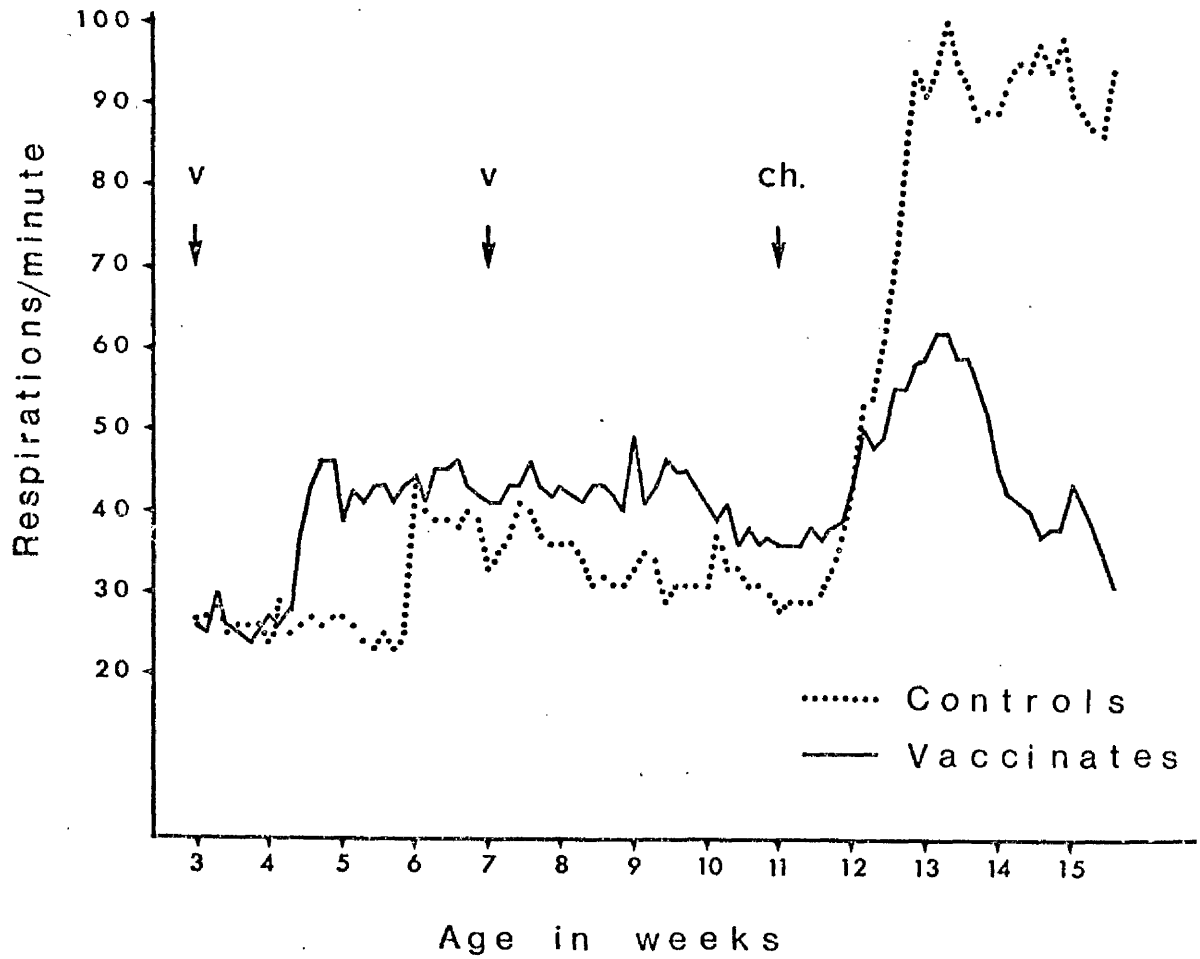
	Vaccinates			Control	
	BW	ZnSO4		BW	ZnSO4
Calf 1	41	22	Calf 2	39	8
" 3	43	30	" 4	36	27
" 5	37	27	" 7	40	7
" 6	43	33	" 8	39	44
" 9	45	19	" 10	43	25
" 12	40	29	" 11	43	18
Mean	41	26.7		40	21.5
S.E.	1.1	2.1		1.1	5.6

challenge the vaccinated animals progressed normally, with the exception of calf 9 which became anorexic and died. The control calves on the other hand showed anorexia from the third week onwards and their condition deteriorated gradually.

Respiratory rates

The mean respiratory rates of the vaccinated and control calves are shown in Fig. 6 and the individual rates are given in Appendix A, Table 6. At 9 days following the first vaccination there was an increase in the mean respiratory rates from 26/min to 46/min; thereafter the rate remained relatively constant until just prior to challenge when the mean was 36/min. At 7 days after challenge the rate increased again reaching a mean value of 62/min during the third week. Afterwards the rate gradually fell to reach 31/min, a level similar to that recorded at pre-vaccination. In the control group the mean respiratory values remained at the accepted normal level of 25/min for the first 3 weeks. At the end of the third week there was a marked increase in the respiratory rates of the control calves to a mean of 43/min; between the third week and challenge 7 weeks later the mean rate fell gradually to 28/min. Starting one week after challenge there was a dramatic increase in the respiratory rate to a mean in excess of 90/min which was maintained for the rest of the experiment.

Figure 6 Mean respiratory rates of 6 groups of suckled calves. Animals of Group 1 were vaccinated at 3 and 7 weeks-old and challenged 4 weeks later together with the control Group 2.



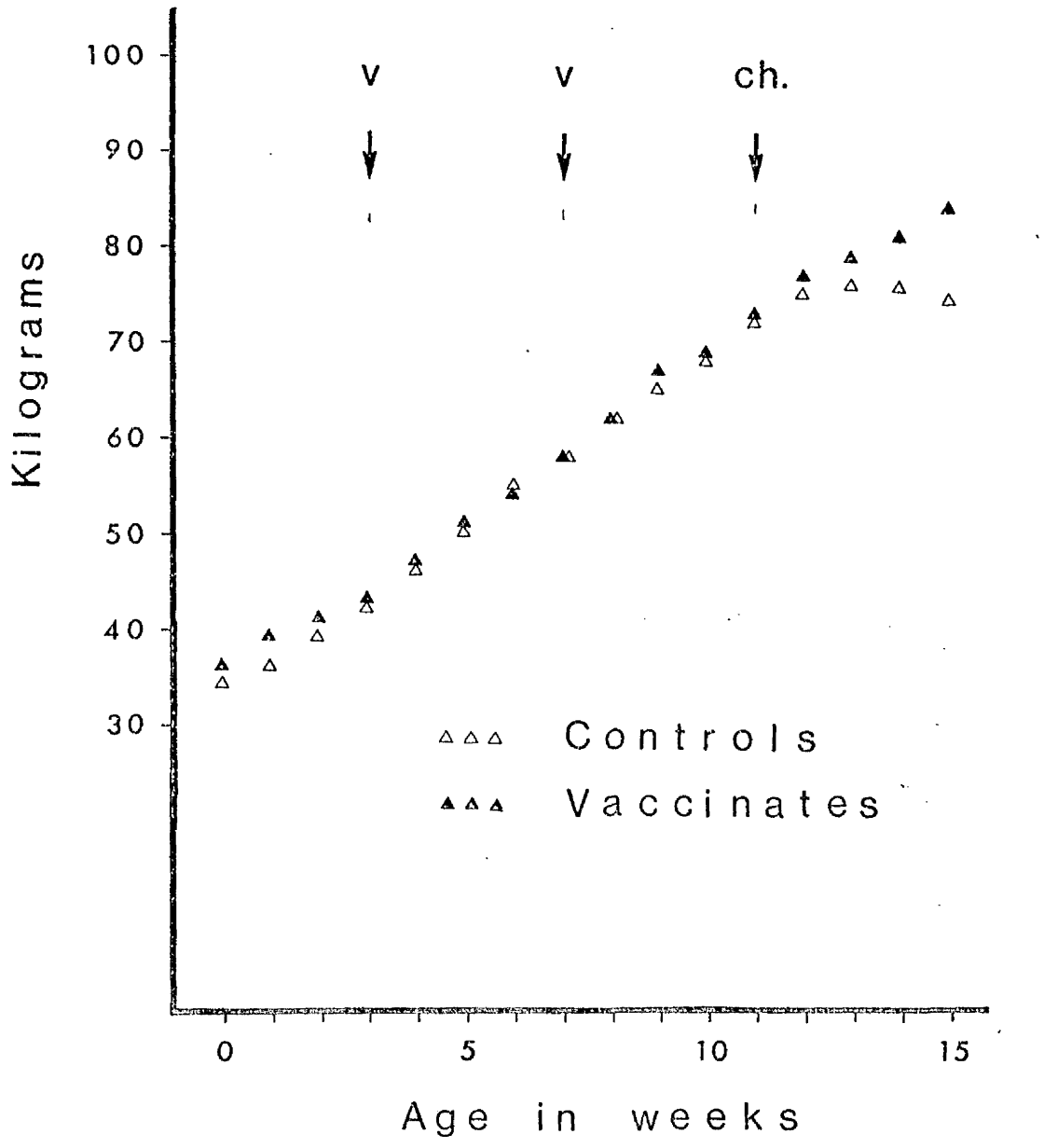
Three calves (a vaccinate No. 9 and controls Nos. 10 and 11) died or were killed *in extremis* following challenge. The vaccinated calf which had an increased respiratory rate from 2 weeks after the first vaccination died on week 13, that is 2 weeks after challenge; of the 2 controls, calf 11 had an elevated respiratory rate prior to challenge and died 2 weeks after challenge. At the same time another calf had to be killed *in extremis*. Bacteriological studies revealed that calf 10 was infected with *Acholeplasma laidlowie* and *Pasteurella haemolytica* and calf 11 with *A. laidlowie*, *Mycoplasma bovirhinis*, *M. dispar*, (*T.* strain ureaplasma). All 3 calves had pneumonia and emphysema.

The vaccinated calves coughed occasionally during the vaccination period as well as following challenge. The control group first coughed one week after challenge, and this increased in severity over the next 3 weeks. Ronchi were present in the control calves but rales were again absent.

Body Weights

The mean body weight of the vaccinated and control calves are shown in Fig. 7 and individual values in Appendix A, Table 7. All vaccinated animals maintained a steady increase in body weights until the end of the experiment. The control group on the other hand started losing weight from the third week and this downward trend was maintained until sacrifice.

Figure 7 Mean body weights of 2 groups of 6 suckled calves. Group 1 calves were vaccinated at 3 and 7 weeks-old and were challenged 4 weeks later together with the control Group 2.



Parasitological Data

Faecal examinations

The faeces of all vaccinated calves were negative for D. viviparus larvae during the vaccination period. Following challenge only one animal gave a positive count of 50 L_1/g during the 4th week. The calves in the control group also became positive at this time, and had a mean of 487 L_1/g. The larval counts per gram are shown in Appendix A, Table 8.

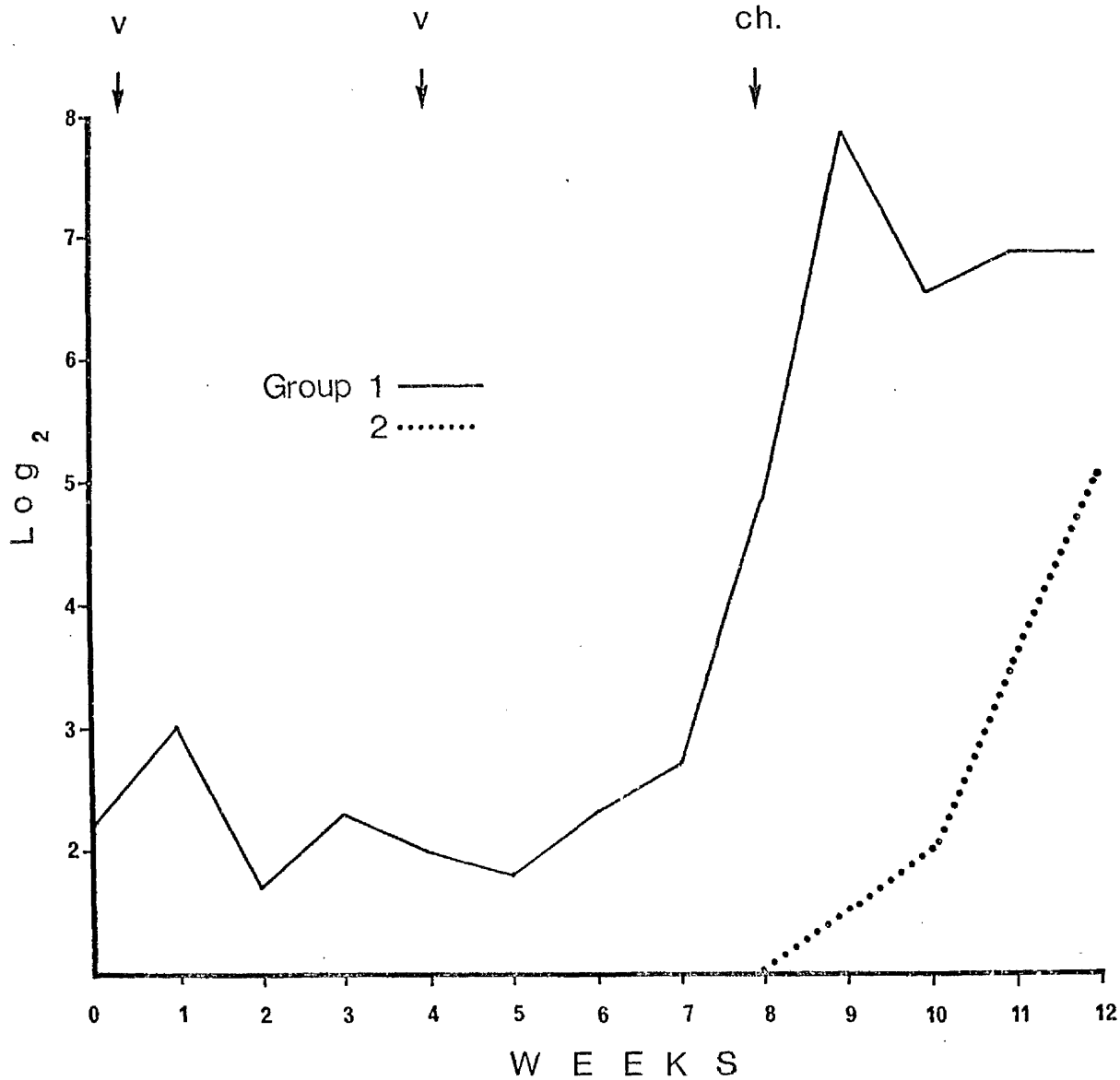
Serological Results

The mean haemagglutination titres of the vaccinated calves (Group 1) and the control (Group 2) are shown in Fig. 8. The mean titres of the vaccinated calves remained constant until week 7, i.e. 3 weeks after the second vaccination and then increased sharply over the next 2 weeks; challenge of these calves took place on week 8, i.e. one week after the increase in antibody titre took place and the peak titre was reached at week 9 and was 7.8 ± 0.7. Following a slight drop to 6.5 ± 0.9 the mean titre was maintained around this value until the experiment was terminated on week 12.

The mean titres of the control group increased following challenge and reached a mean value of 5.0 ± 0.4 just prior to slaughter.

Individual titres are given in Appendix A, Table 9.

Figure 8 Mean haemagglutination titres of 2 groups of 6 suckled calves. Calves of Group 1 were vaccinated at 3 and 7 weeks of age and challenged 4 weeks later together with the control Group 2.



Post-mortem Data

Lung lymphoid nodules

The mean number of lymphoid nodules counted on the surface of the lungs of vaccinated animals was 99, while the controls had a mean of 8. Individual values are shown in Table 5.

Worm recoveries

The number of *D. viviparus* recovered are shown in Table 5. In the vaccinated group the numbers of worms ranged from 4 to 422, with a mean of 142, while in the control group 297 to 1,693 worms were found, the mean being 1,095.

TABLE 5

A comparison of the resistance to experimental challenge of two groups of calves, one of which was previously vaccinated with Dictol while suckling at 3 and 7 weeks of age.

Group	Calf Number	Challenge Dose L_3	*D. viviparus* established at p.m. on day 30 Recovery	% Reduction in worms recovered compared with controls	Lymphoid nodules
1	1	4,740	4	99.6	96
	3	4,500	16	98.5	112
Vaccinates	5	4,170	312	71.5	74
	6	5,250	4	99.6	121
	9	4,680	422	61.5	63
	12	4,740	92	91.6	129
Mean		4,680	142	87	99
S.E.		144.3	73.9	6.7	10.7
2	2	4,560	1,693		5
	4	4,860	991		12
Controls	7	4,650	297		10
	8	4,500	1,168		5
	10	4,830	1,489		Impossible to count due to emphysema
	11	4,230	930		—
Mean		4,605	1,095		8
S.E.		95.1	199.4		1.8

DISCUSSION

These studies were designed to provide information on the efficacy of the immunisation of young calves, i.e. those below the recommended age of 8 weeks, to vaccination with Dictol.

In the first experiment the effect of a challenge dose of D. viviparus larvae on 2 groups of calves, reared on milk substitute and vaccinated with Dictol either at 3 and 7 weeks old or 8 and 12 weeks old was compared with that of non-vaccinated controls.

During this experiment some clinical reaction to vaccination occurred in the younger calves the mean respiratory rates reaching 59/min and 58/min following the first and second vaccination respectively. The older vaccinated calves reacted less severely the mean respiratory rates not exceeding 46/min. Following challenge, the reaction of the vaccinated calves was relatively mild, the mean maximum respiratory rate of the younger calves being 58/min and those of the older being 48/min. In contrast, both control groups of calves reacted severely following challenge and respiratory rates in excess of 90/min were recorded in both groups together with severe respiratory distress.

The body weight gains of vaccinates were not affected by vaccination and although some reduction in weight gain occurred after challenge there was no loss of bodyweight such as occurred 3 weeks after challenge

of the younger control calves. In the older control calves (Group 4) there was a decrease in body weight in the first week after challenge, during the second week the body weight stabilised and then decreased again from the third week onwards until the end of the trial.

At four weeks after challenge lungworm larvae appeared in the faeces of the control calves, while those of the vaccinates remained negative throughout the experiment.

These clinical findings were mirrored in the comparative post-mortem results. Thus the mean lungworm counts in the two vaccinated groups were 28 (Group 1) and 30 (Group 3) while those of the control groups were 861 (Group 2) and 2,789 (Group 4). The mean counts of pulmonary lymphoid nodules in the vaccinates was 141 (Group 1) and 173 (Group 3); in the controls they were 2 (Group 2) and 4 (Group 4).

Three interesting facts emerge from the results of Experiment 1:

First, it is clear that the protection against D. viviparus engendered by Dictol in very young calves, i.e. at 3 and 7 weeks old was as good (96.7%) as that stimulated in older calves, i.e. at 8 and 12 weeks old (98.9%), and age per se does not apparently influence the outcome of the immunisation.

Secondly, the administration of Dictol to young calves did not markedly affect their body weight gains.

This is in contrast to the findings of Downey[44] who reported that calves vaccinated at 7-8 weeks old subsequently made lower body weight gains than unvaccinated controls, although following challenge the vaccinates made superior weight gains when compared with controls[44,45].

Thirdly, the fact that a greater proportion of the challenge dose became established in the control calves of the older group (46.5%) than in the younger group (19.9%). The reason for this discrepancy is not known.

In the second experiment young calves were double vaccinated with Dictol as before, i.e. at 3 and 7 weeks old. At this time of vaccination the calves were suckling foster cows, whereas in Experiment 1 they were fed milk substitute. These animals, together with non-vaccinated suckling calves of a similar age were challenged with normal D. viviparus larvae at four weeks after the second vaccination. In this experiment some reaction to vaccination with Dictol occurred in all of the calves as exemplified by an increase in respiratory rate, although no adverse change in live weight gain and appetite occurred. Following challenge, a more severe reaction occurred in the vaccinates than in the first experiment, the mean respiratory rate reaching 62/min; this however, was not as severe a reaction as occurred in the controls in which the mean respiratory rates reached 100/min, and the severity of the reaction resulted in the death of

two of the controls. One vaccinate which exhibited severe distress had to be sacrificed.

At post-mortem the mean lungworm count in the vaccinates was 142 compared with 1,095 in the controls, while the mean number of lymphoid nodules present were 99 and 8 respectively. The mean reduction in lungworm burden achieved was 87%, i.e. 9.7% less than the calves of a similar age vaccinated in Experiment 1.

Following a first vaccination with Dictol, there were no significant increases in the titres of haemagglutinating antibodies (Figs. 5 and 8). However, after a second vaccination the titres of all vaccinated calves increased; in some instances this increase was maintained until the calves were challenged 4 weeks later, whereas in others the titres fell prior to challenge. Following challenge the titres of all vaccinated calves again increased. A similar trend was present in the control calves after challenge although the maximum level of titre attained was lower than in the vaccinates. A similar pattern of serological response has been noticed in previous experiments[26,54], using the complement fixation test.

Although the overall response of the calves in the different groups were similar some minor differences are apparent. Thus, the mean titre reached by the older

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vaccinated calves of Group 3 in Experiment 1 (Fig. 5), although initially slow to increase, reached a higher level than did that in the younger calves of Group 1.

Also, the reaction of the pail-fed calves in Group 1, Experiment 1 to vaccination was quicker than that of the suckled vaccinated calves in Experiment 2 (Fig. 8). This difference may be due to a possible influence of maternal antibody in the colostrum delaying the onset of active globulin synthesis in the suckled calves, whereas this synthesis occurred rapidly in the pail-fed calves.

Finally, no significant relationship could be found between the antibody titres and the level of resistance shown by vaccinated calves and controls as measured by the lungworm burden at post-mortem. However, a significant difference in the level of antibody titres was present during the first 2 or 3 weeks after challenge.

There are several possible reasons why the degree of immunity produced in the young calves in Experiment 2 was less than that in Experiment 1.

First, at post-mortem the distribution of pneumonic lesions was different, occurring in both apical and diaphragmatic lobes in Experiment 2, whereas the latter area was predominately affected in the calves of Experiment 1. This suggest the presence of some other concurrent infection in the calves of Experiment 2, a fact which was confirmed by bacteriological investigation. It is interesting that mycoplasmas were prevalent in the

calves of Experiment 2 since this group of organisms, apart from their innate pathogenecity, have been reported as causing immunosuppression[56] and this may have contributed to the poorer vaccination response in the calves.

Secondly, the calves in the first experiment were born in January, a time of year when the majority of dairy cows are housed in the UK and are not under challenge from D. viviparus larvae, and the likelihood of significant quantities of antibody being transferred via colostrum from these cows was therefore negligible. In contrast, the calves in the second experiment were born in July, a month when high pasture levels of D. viviparus larvae are frequently recorded[57] and colostral transfer of antibody was a definite possibility. The high immunoglobulin level in the calves of this experiment (as indicated by the Zinc Sulphate turbidity test) certainly indicates that these calves had ingested colostrum.

The significance of the transfer of maternal antibodies in relation to parasitic bronchitis is not known, but studies on the levels of immunoglobin in the calf have shown that the relative levels of immunoglobin both passively derived and actively synthesised are influenced by a) the rate of catabolism of the former, and b) the rate of development of active immunity[58,59,60].

Furthermore, the onset of active synthesis is delayed in calves which ingest large quantities of colostrum and is promoted in colostral deficient calves[61]. In the second experiment at least, the calves had high globulin levels when purchased and it is possible that at 3 weeks of age when vaccination commenced the maternal antibody was largely catabolised (the half-lives of IgE, IgM and IgG are 2, 4 and 21 days respectively[59]) and active synthesis was still sub-optimal; thus the response to vaccination would be impaired and this possibly associated with the postulated immunosuppressive effect of the mycoplasmas present, might account for the reduction in immunity obtained in this experiment.

In both experiments the mean numbers of macroscopic lymphoid nodules in the vaccinated calves following challenge were 141, 173 and 99, whereas in the 3 control groups the mean numbers were 2, 4 and 8. These figures are similar to those obtained by Pirie et al[55], in their studies on the duration of immunity following the administration of Dictol to calves. Jarrett and Sharp[62] have suggested that the pulmonary lymphoid nodules are formed around dead lungworm larvae and are the site of an immunological reaction and as such are a criterion for monitoring the immune status of the calf in relation to D. viviparus. Certainly Pirie and his colleagues[55] found that the number of lymphoid nodules present in the calves prior to challenge was related to the number of vaccination doses administered and the duration of time

elapsing since vaccination. They also found that calves with the highest numbers of these macroscopic nodules after challenge had the lowest number of lungworms. This was borne out in the present studies where the 2 vaccinated calves (5 and 9) with the lowest number of nodules had the highest worm burdens.

The wide ranging studies of Jarrett et al[36,37]; Cornwell[63]; Michel and Mackenzie et al[54], have shown that, when D. viviparus larvae are irradiated at 40 Kr as in Dictol, a few larvae may reach the lung but these never mature to reach the egg laying stage. The consistency of these results made the inclusion of vaccine controls in the current studies unnecessary, a fact confirmed by the absence of larvae in the faeces of all vaccinates prior to challenge. However, the presence of a few lungworm larvae in the faeces of one calf after challenge confirms the findings of Jarrett et al[36,37] and Cornwell[42,63] and Cornwell and Berry[43], and fully justifies the recommendation that vaccinated and non-vaccinated animals should not be grazed together.

Although the present results demonstrate that a highly significant immunity develops following vaccination of young calves with Dictol under experimental challenge, it will be necessary to study the immunity achieved under natural challenge before altering the current recommendation for field vaccination.

Subject to the successful completion of the latter, the most practical application of these findings is the knowledge that suckling beef calves may be safely vaccinated

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with Dictol from 3 to 4 weeks onwards; they should therefore have acquired a solid immunity prior to the ingestion of a significant quantity of lungworm infected herbage. Also, spring born dairy calves could be vaccinated prior to the current recommended age of 8 weeks, and thus allow these calves an opportunity to graze during late summer and autumn.

Apart from the benefits to vaccination programmes in Europe a practical technique of immunising young calves on beef ranches could have a dramatic effect in many tropical countries with a ranching husbandry system and a lungworm problem.

SUMMARY

In these experiments the immunisation of young milk-fed calves with 2 doses of the commercially available Dictyocaulus viviparus irradiated larval vaccine (Dictol) was studied.

Immunisation of pail-fed milk calves at 3 and 7 weeks of age proved highly successful. When these calves and parasite-naive controls also aged 11 weeks were challenged with normal larvae at 66 larvae/kg body weight the controls had a mean of 861 lungworms and the vaccinates 28, i.e. a reduction of 96.7%. Although an increase in respiratory rate occurred following vaccination, the clinical response of the vaccinates in terms of respiratory rates, body weight loss was minimal compared with the controls. The serological titres of the vaccinates were also higher and a higher number of lymphoid nodules were present in the lungs at post-mortem.

Weaned calves aged 8 and 12 weeks and immunised at the same time showed a minimal reaction to vaccination and a reduction in lungworm burden of 99% compared with controls of the same age.

When immunisation of suckling calves was attempted, also at 3 and 7 weeks of age, the immunity developed was poorer than in the pail-fed calves. The mean worm burden of vaccinates was 142 and that of the controls 1,095, i.e. a reduction of 87%. Also a more severe clinical reaction was noted in these vaccinated calves in terms of respiratory

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rates and weight loss than in the pail-fed vaccinates. The lower immunity may have been induced by a concurrent pneumonia due to Mycoplasma spp or possibly maternal antibody may have blocked the effect of the first immunising dose.

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SECTION 2.

STUDIES ON VACCINATION OF CALVES AGAINST OSTERTAGIA OSTERTAGI

In temperate zones of the world the abomasal nematode Ostertagia ostertagi[1] is the most important gastrointestinal nematode of cattle. Thus, field studies in the U.S.A.[2,3,4,5,6,7,8], in Australia[9,10], Canada[11,12] and in Britain[13,14,15] have demonstrated that O. ostertagi was the dominant species present in outbreaks of parasitic gastroenteritis and experimental studies have confirmed the pathogenic potential of the parasite[14,16,17,18]. In the latter experiments clinical parasitic gastritis characterised by weight loss and diarrhoea and closely resembling the field syndrome was produced by single experimental infections of at least 300,000 infective larvae or repeated infections with lower infecting doses.

As a result of these studies and previous investigations on the taxonomy and evolution of the parasite[1,19,20,21,22], the histopathological changes produced in the abomasal mucosa[23,24] and on the epidemiology of the disease[25,26,27,28,29,30,31] the detailed life-cycle may be summarised as follows:

The adult male and female worms, which measure 8-10 mm in length, lie on the surface of the abomasal mucosa and eggs laid by the fertilised females pass out in the faeces. Under suitable climatic conditions (a mean day/night temperature of $>10^{\circ}C$ and a high relative humidity) the eggs hatch into the sluggish first stage larvae (L_1) which in

turn develop into second stage larvae (L_2) and finally become the actively motile third stage infective larvae (L_3). Most of this development takes place in the faecal pat and provided sufficiently wet conditions exist, such as created by rainfall, the larvae migrate on to the herbage. The infective L_3 stage retains the outer sheath of the L_2 and this extra protective coat enables many of the L_3 to survive over the winter, although the majority of these die by the following summer; in contrast the other free-living stages are susceptible to adverse conditions such as drought and cold and are short-lived.

If ingested by a susceptible bovine the L_3 casts its extra sheath in the rumen, passes into the abomasum and enters the gastric glands where it moults to the fourth larval stage (L_4) about day 4 following ingestion. During the next week the L_4 (1 mm in size) grows and by day 10 moults to the fifth and final larval stage (L_5). A period of rapid growth and sexual maturation ensues and by day 18 these fully mature adults (8 to 10 times the size of the L_4) are ready to leave the gastric glands to reside on the mucosal surface. Under certain conditions the larvae remain arrested in development at the L_4 stage for periods of up to 6 months.

During its sojourn in the gastric glands the parasite provokes cellular changes in the glands[32] and the secretory cells, particularly the parietal HCl

producing cells, are replaced by undifferentiated epithelial cells. At this stage the mass of non-parasitised cells remain unaffected and gastric function is not impaired. However, as the parasite grows and dilates the glands and coincident with the emergence of the adult parasites from the glands, dramatic cellular changes take place in the non-parasitised glands and the parietal cells become non-functional with a resultant loss of acidity in the abomasum. This leads to impaired digestion and increased numbers of viable bacteria in the stomach and diarrhoea.

In view of the prevalence of O. ostertagi infection in dairy farms in Britain and the potential economic losses caused by the disease[33], successful control measures have been sought. Two control schemes have been developed by Michel[26,33] and Leaver[34]. In Michel's scheme calves in their first grazing season are treated in July with an anthelmintic and moved to fresh pastures not grazed in that season by other cattle. This protocol is based on the epidemiological knowledge that the numbers of overwintering L_3 on the first pasture grazed from early spring, will be insufficient to result in clinical disease and that the fresh pasture contamination with eggs which results from this infection will not become L_3 until mid-July at the earliest; the second pasture, grazed from mid-July, will be virtually clear of L_3 by that time due to mortality of the overwintering

population of L_3. This programme has given good control except in years when the numbers of overwintered L_3 are exceedingly high. It has the disadvantages that it caters only for farms with a plentiful supply of alternative grazing and also by limiting the exposure of the calf during the early grazing season the acquisition of a good immunity may be delayed.

In Leaver's system, susceptible calves are rotationally grazed ahead of adult and presumably immune cattle which remove many of the larvae from the herbage and so lower the level of infection available to the calves. While this system has many admirable features it (like Michel's programme) requires a surplus of grazing which is seldom available on the smaller dairy farms where ostertagiasis is endemic.

An immunological approach to the control of ostertagiasis is a much more attractive one, and it is perhaps appropriate at this point to review the existing knowledge on immunity to bovine parasitic gastritis and in particular to ostertagiasis.

Recent studies on immunity to gastrointestinal nematodes of cattle, have concentrated on the part played by the age of the host and acquired immunity; preliminary attempts have also been made to induce an artificial immunity by the administration of X-irradiated larvae. Considerable interest has also centred on the phenomenon of inhibited larval development and its possible

relationship to immunity. It is proposed to consider immunity under these headings.

In the United States, outbreaks of clinical ostertagiasis are known to occur when adult stock are moved from areas where the latter parasite does not occur, to areas where outbreaks are frequently recorded in young stock[6]. It therefore appears that immunity to ostertagiasis is dependent on previous exposure to the parasite rather than to the age of the host. Experimental studies, also in the United States by Herlich[35], using mixed infections of O. ostertagi, Cooperia punctata and Trichostrongylus axei, showed that while adult cattle were more resistant to the debilitating effects of nematodes, age per se did not influence either the pre-patent period,or the numbers or size of the worms established or their egg production; the cattle used in these experiments were 18-25 months old. More recently, in Britain, Armour[36] has demonstrated that following a large single infecting dose (200,000) O. ostertagi there were considerable differences in both the clinical response and parasitological findings between parasite-naive calves aged 6 months and parasite-naive cattle aged 30 months or more; there was a prolonged pre-patent period in the older animals and the female worms, while adult in terms of size, did not contain any eggs. Similar findings have been reported by Smith and Archibald[37] working with natural

infections of C. oncophora in Canada. It would appear, therefore, that an absolute age immunity does not operate against the common gastrointestinal nematodes of cattle including O. ostertagi but may influence the course of infection viz. an extended pre-patent period and a reduced pathogenic effect and possibly an increased ability to acquire immunity.

Acquired immunity to gastrointestinal helminths does occur under natural grazing conditions and has been demonstrated in Australia[38,39] in cattle with a mixed population of nematodes consisting of the stomach worm H. placei, the hookworm Bunostomum phlebotomum and Cooperia spp.; it has also been shown in the United States with C. punctata[40]. In Britain there is evidence from field studies[36,43] that cattle acquire a high degree of immunity to C. oncophora and Nematodirus helvetianus, two of the most common gastrointestinal nematodes present in British cattle. The immunity to these parasites appears to be relatively absolute in that high worm burdens of these species are seldom encountered in adult stock.

With O. ostertagi the situation is less clear as, certainly in Britain, outbreaks of parasitic gastritis are rare in adult stock although they are relatively common in dairy calves. However, this may merely reflect the management practice in dairy areas where productive adult stock are grazed on the best and

frequently new pastures not previously contaminated with eggs and larvae of gastrointestinal nematodes. Alternatively, since the area of the abomasum mucosa in adult animals is up to 6 times that in young calves, it would require massive infestations before sufficient abomasal damage would occur to result in clinical disease.

A third and more probable reason is that adult cattle will have acquired an immunity to O. ostertagi through exposure to infection during their first and second grazing seasons. The acquisition of immunity to O. ostertagi by grazing stock has been studied in Northern Ireland by Ross and Dow[41,42] and in Scotland by Armour[36]. In the former studies[41,42] calves were grazed throughout the summer and early autumn on pastures known to be contaminated with the parasite; at the end of the grazing period these calves and their parasite-free controls of the same age, were experimentally challenged with large numbers of O. ostertagi L_3. In contrast to the controls the previously exposed animals showed no clinical signs of ostertagiasis and at subsequent post-mortem the abomasal lesions were not severe. Ross and Dow therefore concluded that animals grazing on pastures where Ostertagia larvae were endemic, acquired an immunity to this parasite by the end of their first

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grazing season. Further studies by Armour[36] showed that if calves which had grazed for one season on Ostertagia contaminated pasture were then housed during the winter they were not resistant to a subsequent experimental challenge in the following spring. However, although clinical signs of ostertagiasis occurred in these challenged animals they were not of such severity or duration as in worm-free controls of the same age. Also, there was some indication that the worm burdens of the challenged and previously infected calves were more rapidly expelled than by the controls. Armour therefore suggested that during the period of housing, the absence of stimulation by ingested larvae had resulted in a waning of the immunity which had been acquired during the first grazing season and that this was reflected in an increased susceptibility to challenge; on subsequent reinfection in the second grazing season, the immunity was rapidly regained.

Age immunity may also contribute in a modifying fashion by inhibiting the sexual development of the worms and therefore decreasing the production and dissemination of Ostertagia eggs, i.e. the biotic potential of the parasite becomes reduced. Outbreaks of ostertagiasis are therefore unlikely to occur where groups of adult cattle are grazed together in endemic

areas. If however, individual adults are grazed on pastures which have been recently grazed and contaminated by infected young stock, the pasture burden of infective larval stages acquired may be sufficiently high to overcome the host immunity.

Four attempts have been made to immunise cattle against gastrointestinal nematodes using irradiated larvae. The first, in Nigeria[44] was against H. placei and consisted of the administration of two doses of infective larvae previously subjected to ionising radiation. While the vaccinated calves achieved a 70% protection against experimental challenge compared to worm-free controls, no protection was apparent following a naturally acquired field challenge (Armour, Lee and Ross - personal communication).

The second, involved attempts to immunise calves against O. ostertagi[36], also using two doses of X-irradiated larval vaccine at an interval of one month. 10 calves were vaccinated, 5 being challenged experimentally 30 days after vaccination together with 5 worm-free controls. The remaining 5 vaccinates were grazed together with 5 controls on pastures known to be contaminated with O. ostertagi larvae. In the calves challenged experimentally there was no significant difference in the magnitude of the worm burdens at post-mortem, but in the vaccinates the clinical disease was less severe, the faecal egg counts much lower and the pre-patent period extended. In the calves

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challenged naturally by grazing for 16 weeks on the Ostertagia contaminated pasture, 2 deaths occurred from ostertagiasis in the controls and one in the vaccinates. Of the remaining 4 vaccinates 2 were highly immune as judged by comparison of the worm burdens with those of the control calves, and the remaining 2 were apparently susceptible. The other studies on immunisation were carried out in West Germany by Burger, Eckert, Chevalier, Rahman and Konigsmann[45] and Burger and Pfeiffer[46] and their results were similar to those obtained by Armour in 1967[36] in that although some reductions in the numbers of worms and the numbers of eggs in the faeces of individual animals were recorded the differences in overall worm burdens between vaccinates and controls were not significant.

Finally, in any discussion on nematode immunity it is usual to find a section devoted to the arrested development of the parasitic larval stages and since this phenomenon has frequently been ascribed to the development of immunity[47,48,49,50] its possible occurrence would have to be considered in any immunisation programme. Until recently it was generally accepted that as immunity is acquired during the grazing season, the larvae ingested towards the end of the grazing period become arrested in their development. The subsequent development of these arrested larval stages occurred in

the spring of the following year and was considered to be associated with a decline in the immune status of the host during the winter housing period due to the absence of reinfection and therefore antigenic stimulation. In the case of O. ostertagi, if sufficient arrested larvae develop synchronously clinical disease occurred[13]. These theories on the aetiology of arrested larval development and their subsequent maturation may require reinterpretation in view of the recent findings in Glasgow that arrested development of O. ostertagi occurs seasonally in the autumn and independent of the immune status of the host[51,52]. Furthermore, experimental studies by Armour and Bruce[53] have clearly shown that arrested larval development of O. ostertagi can be induced by chilling and that the period during which the larvae remain arrested in their development appears to be of a fixed time interval possibly related to the period of induction.

If the mechanism of arrested development of O. ostertagi larvae in the host is non-immunological then theoretically large numbers of arrested L_4 could accumulate despite the immunity produced following vaccination against this parasite. Initially, this could prove a disadvantage to the use of an Ostertagia vaccine in that clinical disease might supervene; however, the long-term effect of a vaccine on the biotic potential of the nematode would, hopefully, so reduce the level of infection on a farm that the problem created by a few arrested larvae would

be insignificant. Although the preliminary attempts to immunise cattle against O. ostertagi have not been entirely successful, some reduction in egg production of the established challenge infection in the vaccinates occurred. Perhaps, therefore, an immunisation programme using attenuated larvae could be used to gradually lower the level of infection in endemic areas, and act as an adjuvant to chemotherapeutic control methods.

In the current studies the protection against natural challenge achieved by vaccinating calves with γ-irradiated O. ostertagi larvae was studied and the long-term effect of the vaccinated calves on the herbage levels of infective larvae over a two year term was compared with the results obtained by grazing fully susceptible controls.

EXPERIMENT 1 - 1973

Experimental Design.

In March 1973, 6 calves reared parasite-free and aged 8-10 weeks were each given 100,000 O. ostertagi infective larvae which had been γ-irradiated at 60 Kr; one month later the calves were each given a similar treatment. At the same time as the attenuated O. ostertagi larvae were administered, these calves and 6 parasite-free controls were immunised with the commercially produced attenuated D. viviparus larval vaccine, Dictol.

One calf allotted to the control group died from a colisepticaemia and was replaced by another parasite-free calf; unfortunately, this calf (No. 13) was 5 weeks older than the others and therefore considerably heavier.

During April, 4 paddocks each of approximately 0.3 hectares were rotationally grazed by 3 "seeder"calves, 2 of which had been infected with 100,000 O. ostertagi infective larvae at the beginning of March and at the time of grazing were excreting eggs in their faeces. The other "seeder" animal was a clinical case of ostertagiasis obtained from the Medicine Clinic at the Glasgow Veterinary School. In order to equate the degree of contamination of the 4 paddocks the "seeder" calves were rotated from paddock to paddock every second day. These measures were undertaken to ensure a reasonable level of larvae on the pastures during the summer since

the paddocks had not been grazed by calves in the previous year, although prior to then clinical ostertagiasis and dictyocauliasis had occurred.

In May, 3 of the experimental calves were allocated to each paddock which meant that 2 paddocks were grazed by calves immunised against O. ostertagi and D. viviparus and 2 by calves immunised solely against the latter. For brevity, the former groups are called the vaccinates and the latter the controls.

The calves grazed for 24 weeks (unless disease supervened) and were then housed for one week prior to slaughter. The experimental design is summarised in Table 1.

Observations

Prior to vaccination and throughout the housed post-vaccination period the animals were clinically examined each day. At pasture the animals were observed daily and when considered necessary a more detailed clinical examination was carried out. At monthly intervals throughout the experimental period the animals were weighed. Faeces were collected from the rectum at weekly intervals for nematode larval and egg counts; blood samples from the jugular vein were obtained at the same time for biochemical examinations (plasma pepsinogen).

At post-mortem the entire gastrointestinal tract and the lungs were removed and examined for the presence of nematodes as indicated previously.

TABLE 1.

Experimental Design. 1973.

Group	Calf Number	Pre-grazing vaccination: 2 x 1,000 γ-irr. *D. viviparus* L_3 (Dictol*)	Pre-grazing vaccination: 2 x 100,000 γ-irr. *O. ostertagi* L_3+ 2 x Dictol*	Grazing Period
Vaccinates				
2	109	-		10.10
	113	-		24.10
	111	-		7.11
			22.3 & 23.4	21.5 to
3	107	-		7.11
	108	-		7.11
	110	-		7.11
Controls				
1	114		-	10.10
	121		-	7.11
	780		-	7.11
		22.3 & 23.4		21.5 to
4	116		-	12.9
	13		-	7.11
	385		-	7.11

* Dictol - Allen & Hanburys Ltd., Ware, Herts.

During the experimental period herbage samples were collected from the 4 paddocks and processed and analysed for the presence of nematode larvae.

Results

Clinical Data

In both vaccinated and control groups, clinical signs of ostertagiasis, namely diarrhoea, first appeared in the middle of August. Diarrhoea continued intermittently in all of the animals until the end of the grazing season. In the vaccinates the clinical signs were so severe that 2 animals (109 and 113) were killed *in extremis* during October; in the controls, calves 114 and 116 were similarly affected and were also slaughtered. The severity of the diarrhoea is classified in Appendix B, Table 1.

About the middle of September several calves showed evidence of increased respiratory rates and coughing. 3 of the vaccinated group (Nos. 108, 109, 113) had respiratory rates which fluctuated between 60 and 80 and 2 of the control calves (Nos. 114 and 116) were similarly affected.

The mean liveweight changes of the calves in each of the 4 groups are plotted in Figure 1 while the individual values are given in Appendix B, Table 2.

During the period of housing, prior to grazing, all the animals steadily gained weight. On going to pasture, some reduction in weight gain was noted in one control

Figure 1 Mean body weights of 4 groups of 3 calves which grazed on O. ostertagi contaminated pasture during 1973. Groups 2 and 3 were vaccinated with 2 x 100,000 γ-irradiated O. ostertagi L_3 and 2 x Dictol prior to grazing; Groups 1 and 4 received only 2 x Dictol.

group (Group 1) and in one of the vaccinated (Group 2); the other vaccinated and control groups (3 and 4) gained weight steadily until September. From the end of September onwards the weight gains of surviving calves in both control groups increased steadily, whereas the surviving vaccinates, except calf No. 111 in Group 2, lost weight.

Biochemichemical Data

Plasma pepsinogen levels

The mean levels of plasma pepsinogen of all groups expressed as mU of tyrosine are shown in Fig. 2 and individual values are presented in Appendix B, Table 3.

Following the administration of the 2 doses of 100,000 O. ostertagi larvae the mean level of plasma pepsinogen increased in both vaccinated groups. In Group 2 the level reached a mean maximum of 1,600 mU following the second vaccination, while calves in Group 3 exceeded a mean maximum of 2,000 mU. During the initial grazing period the level of all vaccinated calves dropped again to approximately 1,000 mU. After one month of grazing the mean levels steadily increased in both vaccinated groups reaching a mean maximum of 5,900 mU in Group 2 and 7,000 mU in Group 3. The control calves showed a similar pattern, the levels of plasma pepsinogen being normal (<1,000 mU) during the first month of grazing and increasing thereafter to a mean maximum of 6,200 mU in Group 1 and 5,700 mU in Group 4 by September-October.

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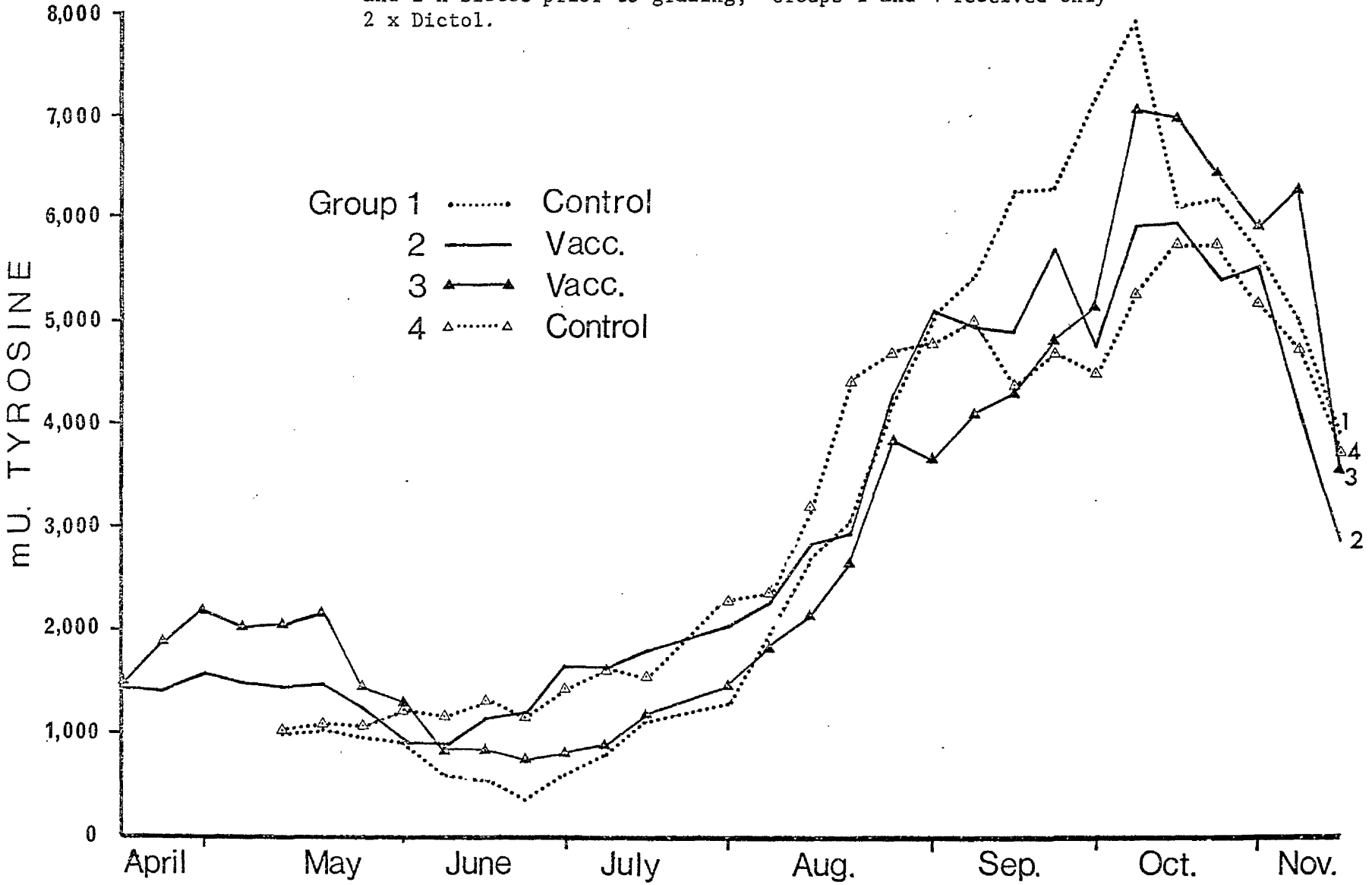
Figure 2 Mean plasma pepsinogens of 4 groups of 3 calves which grazed on O. ostertagi contaminated pasture during 1973. Groups 2 and 3 were vaccinated with 2 x 100,000 γ-irradiated O. ostertagi L_3 and 2 x Dictol prior to grazing; Groups 1 and 4 received only 2 x Dictol.



By the end of the experiment the mean levels of plasma pepsinogen in both vaccinates and controls decreased dramatically.

Parasitological Data

Faecal egg and larval counts

The mean trichostrongyle faecal egg counts are plotted in Fig. 3 while the individual counts are presented in Appendix B, Table 4. The faeces of all calves were negative for trichostrongyle eggs prior to grazing; after 15 days at pasture 50 eggs per gram (epg.) were noticed in the faeces of two of the calves vaccinated with attenuated O. ostertagi larvae. By one month after grazing some of the calves in each group were excreting trichostrongyle eggs. From early August a marked increase in the mean faecal egg counts of all the groups occurred reaching a mean maximum of 4,333 epg. in the control group 4 during September. This was due to a very high epg. of calf 116, which subsequently died. At housing in November, the number of trichostrongyle eggs in the faeces of the surviving calves in all groups had dropped considerably and remained low, except in those of vaccinated Group 3.

A few Nematodirus spp., Strongyloides papillosus eggs and Eimeria spp. oocysts were noticed from time to time and these are shown in Appendix B, Table 5.

D. viviparus larvae were excreted by some calves in each group during the latter half of the grazing season, reaching 600 larvae per gram in one instance. Individual

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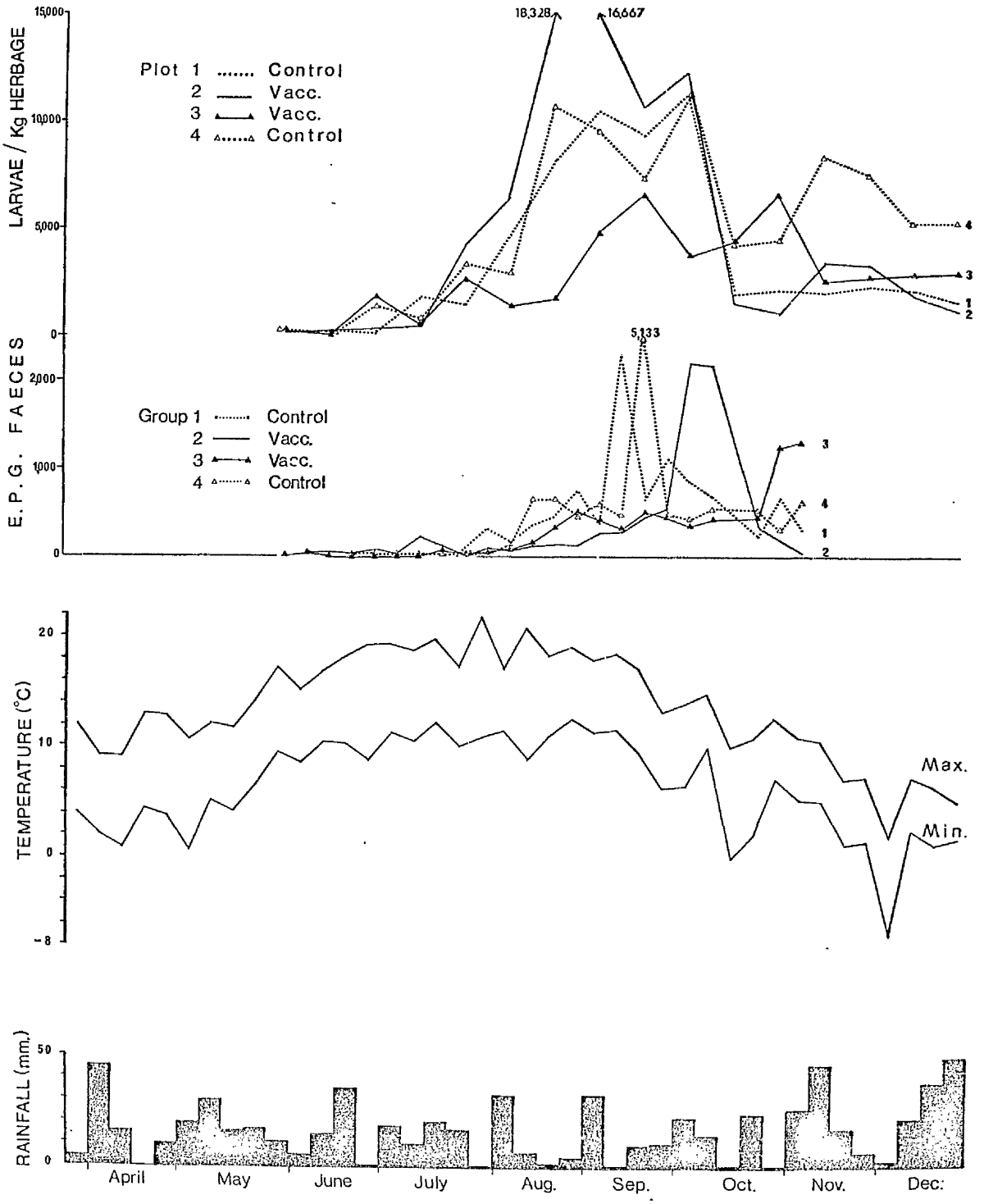
Figure 3 Details of temperature and rainfall in 1973, pasture larval counts of O. ostertagi L_3 from 4 plots grazed by separate groups of calves and mean trichostrongyle faecal egg counts from these calves (Groups 2 and 3 were vaccinated against O. ostertagi and D. viviparus; Groups 1 and 4 only against D. viviparus).



counts are given in Appendix B, Table 5.

Worm recoveries

The individual numbers of nematodes recovered at post-mortem of the calves are shown in Table 2. The individual O. ostertagi worm counts are expressed as: total worms, males, females and inhibited L_4, which are also expressed as a percentage of the total. The other gastrointestinal nematodes are grouped together under the heading 'other trichostrongyles'. Individual numbers of 'other trichostrongyles' are given in Appendix B, Table 6. The number of lungworms present in each of the calves at post-mortem is also shown in Table 2.

By analysis of variance there were no significant differences in the total numbers of O. ostertagi present at post-mortem of the different groups. Details of the statistical analysis are given in Appendix B, Table 7. The numbers of female worms present at post-mortem consistently exceeded the numbers of males. The mean ratio of males: females for vaccinated calves and controls was 1:1.3. In the calves which died prior to November the percentage of inhibited larvae in the worm population ranged between 3.4 in September and 65.9 in October; in those calves slaughtered in November the mean was 78.4 to 97.1.

At post-mortem lungworms were present in 9 of the 12 calves, all of which had previously been vaccinated with

TABLE 2.

Gastro-intestinal and lungworm counts at post-mortem of 4 groups of calves grazed on naturally contaminated pastures during 1973; prior to grazing 2 groups (2 & 3) were immunised against Ostertagia ostertagi and Dictyocaulus viviparus with irradiated larvae and 2 groups (1 & 4) only against lungworm.

Group	Calf Number	Date of Death	Ostertagia ostertagi Total	Male	Female	L_4	% L_4	Other Tricho-strongyles*	Lung-worm
Vaccinates									
	109	10.10	86,300	15,900	18,900	51,500	59.7	6,400	448
2	113	24.10	66,500	19,300	28,000	19,200	28.9	10,500	31
	111	14.11	53,600	4,400	6,800	42,400	79.1	1,300	3
	Mean		68,800			37,700	55.9		
	S.E.		9,509			9,616	15.0		
	107	14.11	106,800	8,100	11,300	87,400	81.8	1,300	5
3	108	14.11	108,200	11,300	12,100	84,800	78.4	25,800	135
	110	14.11	220,200	14,700	18,800	186,700	84.8	19,100	-
	Mean		145,067			119,633	81.7		
	S.E.		35,569			33,542	1.8		
Controls									
	114	10.10	185,200	26,300	36,900	122,000	65.9	23,800	253
1	121	14.11	214,200	4,200	7,400	202,600	94.6	18,800	-
	780	14.11	77,000	5,900	7,000	64,100	83.2	16,600	4
	Mean		158,800			129,567	81.2		
	S.E.		41,748			40,160	8.3		
	116	12.9	119,100	53,900	61,200	4,000	3.4	2,800	53
4	13	14.11	79,600	700	1,600	77,300	97.1	1,600	-
	385	14.11	210,700	15,500	13,800	181,400	86.1	25,700	5
	Mean		136,467			87,567	62.2		
	S.E.		38,829			51,468	29.6		

* Include nematodes of the genera Trichostrongylus, Cooperia and Nematodirus.

Dictol. In 3 of the calves, 2 which had also been previously vaccinated with O. ostertagi larvae, the numbers of lungworms present were particularly high being 135, 253 and 448.

Pasture larval counts

Nematode larvae could not be detected on the herbage of any of the 4 paddocks until the end of May; thereafter O. ostertagi and a little later Cooperia oncophora, Nematodirus helvetianus and D. viviparus third stage larvae were present in the herbage samples. N. battus larvae were found occasionally.

The numbers of O. ostertagi L_3 recovered from each paddock at fortnightly intervals and expressed as larvae per kilogram of dried grass are shown in Fig.3 and Appendix B, Table 8. Fig.3 also includes the mean trichostrongyle faecal egg counts and the appropriate climatic data. From July a massive increase occurred in the number of L_3 present (up to a maximum of 18,328) in plot 2 grazed by the vaccinates and a considerable rise also occurred in plots 1 and 4 (up to a maximum of 11,000 L_3). In the remaining paddock, namely No. 3, grazed by vaccinated calves, the flush of a significant number of L_3 did not occur until September, when the level reached only 4,000-5,000 L_3. During October, a marked drop in the numbers of L_3 occurred in all the paddocks and the level remained between 1,000-3,000 L_3 until the end of the year, with the exception of paddock 4 in which over 5,000 L_3 were present during December. The number

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of C. oncophora and Nematodirus spp. L_3 on the herbage samples were also recorded and are shown in Appendix B, Table 9. By comparison with the number of O. ostertagi L_3 present in the herbage, only low levels of infection with these species were found. The maximum number of C. oncophora recorded was 2,563 from paddock 1 during September and only one high count of Nematodirus spp., namely 3,333 was recorded in November from paddock 4.

The numbers of D. viviparus L_3 from each paddock are recorded in Appendix B, Table 9. This species was first noticed in the herbage samples during September and was present in only low numbers; by the end of November no lungworm larvae were recovered from the herbage samples.

Climatic Data

The mean weekly maximum and minimum temperatures and the weekly rainfall are also shown in Fig. 3 together with the faecal egg counts and the herbage samples. During 1973, the climatic data was within that usually observed in the West of Scotland.

Post-mortem Data

the pH of the abomasal contents of the animals at post-mortem are shown in Table 3. The results from the animals which died in the field have not been considered since these animals were not subject to post-mortem examination within 30 minutes of death. In the animals where data was available within 30 minutes of slaughter, the pH values

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recorded were consistently above the accepted normal value of 2-3; these changes were most marked in the vaccinated Group 3 where the mean pH value was 4.9 and in control Group 4 where the mean was 5.1 and in which calf 385 had a pH of 6.4.

On gross examination of the abomasa of the experimental animals, lesions consistent with those of ostertagiasis as described by Armour[36] were present. These included nodules, areas of nodule coalescence and in the more severely affected calves some mucosal congestion and oedema. These lesions were consistently present in both vaccinates and controls. The abomasum of calf 385 is shown on Plate 1 and that of calf 108 is shown on Plate 2.

TABLE 3.

pH of the abomasal contents at post-mortem of calves grazed on Ostertagia contaminated pastures during 1973; prior to grazing some calves (Groups 2 & 3) were vaccinated with γ-irradiated O. ostertagia and D. viviparus larvae. The controls were immunised against D. viviparus only.

Group	Calf Number	pH
Vaccinates		
2	111	3.5
3	107	4.7
	108	4.5
	110	5.4
	Mean	4.9
Controls		
1	121	3.5
	780	4.5
	Mean	4.0
4	13	3.8
	385	6.4
	Mean	5.1

PLATE 1 Abomasum from a control calf (No. 385) showing lesions characteristic of ostertagiasis.

PLATE 2 Abomasum from a vaccinated calf (No. 108). The nodule formation is less developed than the control calf above.

EXPERIMENT 2 - 1974

Experimental Design

In 1974 the protocol for the experiment was similar to that of 1973 although some enforced changes in the design took place during the course of the experiment. The latter were influenced initially by the poor weight gains of the calves during the pre-experimental rearing period and subsequently by an outbreak of mucosal disease while the calves were at pasture. As a result the calves were not vaccinated until April and May and grazed from the 9th June, i.e. almost a month later than in 1973. The vaccinates grazed in the same paddocks 2 and 3 as did the vaccinates in 1973 while the controls again grazed in paddocks 1 and 4. Due to mucosal disease 3 out of the 12 calves under experiment died in July; the surviving animals in the two vaccinated and two control groups were then amalgamated into single groups and grazed alternatively in the appropriate two paddocks. Only data pertaining to the surviving calves have been included in the results. The experimental design is summarised in Table 4.

Observations

The same procedures and observations were carried out over 1973 during this experiment in relation to 1973.

Results

Clinical Data

All of the calves developed a severe diarrhoea about 10 days after commencing grazing. The diarrhoea was

TABLE 4.

Experimental Design. 1974.

Group	Calf Number	Pre-grazing vaccination: 2 x 1,000 γ-irr. *D. viviparus* L_3 (Dictol*)	Pre-grazing vaccination: 2 x 100,000 γ-irr. *O. ostertagi* L_3+ 2 x Dictol*	Grazing Period
Vaccinates				
	885	-		18.9
	887	-		18.9
	204	-	17.4 & 17.5	9.6 to 29.10
	238	-		29.10
	903	-		29.10
Controls				
	196		-	29.10
	881		-	29.10
	888	17.4 & 17.5	-	9.6 to 29.10
	891		-	29.10

* Dictol - Allen & Hanburys Ltd., Ware, Herts.

characteristic of mucosal disease being greyish in colour and foul smelling and quite unlike the usual bright green faeces associated with either the nutritional effect of the lush grazing or ostertagiasis.

On clinical examination ulcers typical of mucosal disease were present on the lips and palate (Plate 3). After another 2 weeks the diarrhoea decreased in severity in the surviving calves and became intermittent in occurrence. As a result of this outbreak, 2 control and 1 vaccinated calves died and were excluded from the results.

Towards the end of the grazing period, i.e. in September, an increase in the respiratory rates of 2 of the vaccinated calves occurred to between 60 and 80 per minute and this was accompanied by a husky cough, symptomatic of parasitic bronchitis. At the same time a mild diarrhoea in which the faeces were bright green was present in one of the vaccinates and 3 of the control calves. Details of the pattern of diarrhoea is given in Appendix B, Table 10.

The mean body weight gains of the surviving 5 vaccinates and 4 controls are presented in Fig. 4. Individual weights are shown in Appendix B, Table 11. Initially the vaccinated calves were lighter than the controls but this trend was reversed during the grazing period, and at the final weighing the principals had a mean body weight of 137 kg., compared to 125 g of the controls.

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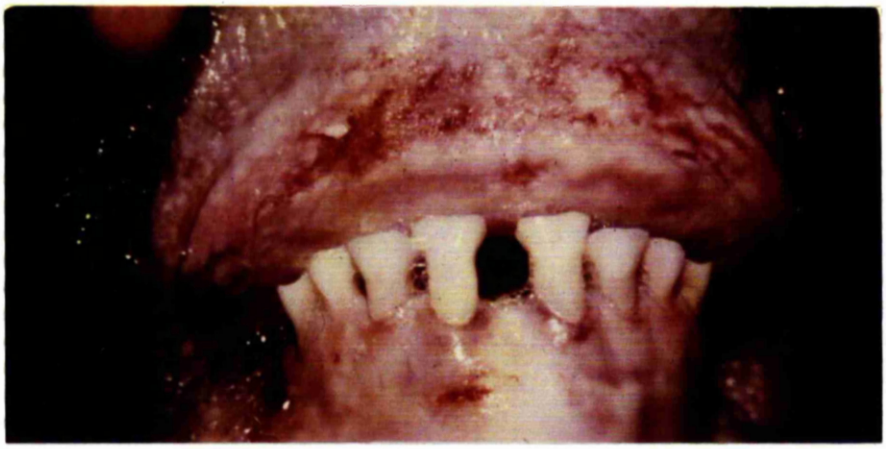
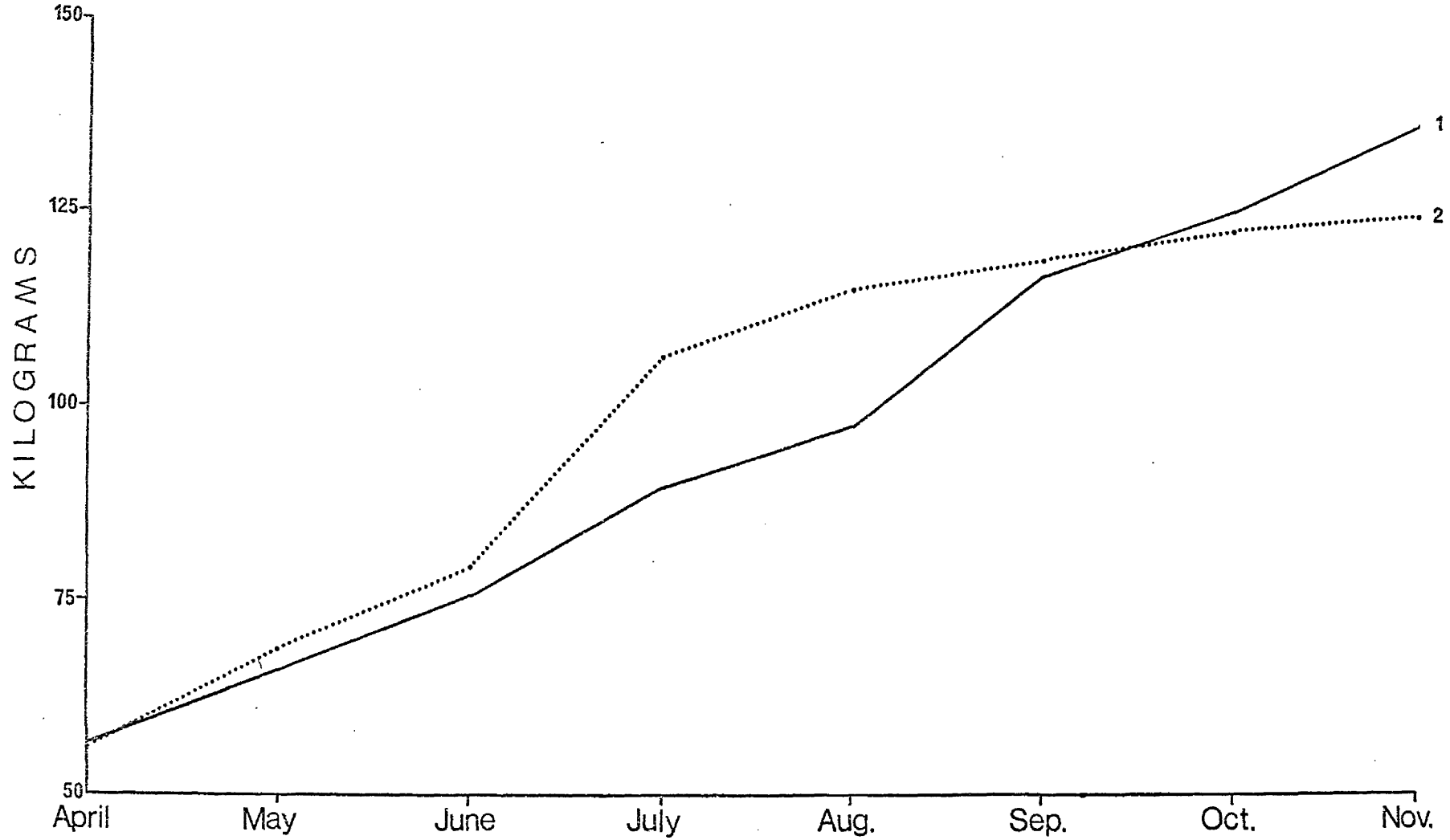


PLATE 3 Mouth of an animal presenting typical lesions of mucosal disease.

Figure 4 Mean body weights of 2 groups of calves which grazed on O. ostertagi contaminated pasture during 1974. Group 1 was vaccinated with 2 x 100,000 γ-irradiated L_3 and 2 x Dictol prior to grazing; Group 1 received only 2 x Dictol.



Biochemical Data

Plasma pepsinogen levels

The mean levels of plasma pepsinogen of both groups are presented in Fig. 5 and individual values in Appendix B, Table 12.

Following administration of the first dose of γ-irradiated O. ostertagi L_3 the level of plasma pepsinogen increased reaching a mean of 2,000 mU about 3 weeks after vaccination and then declined slowly. Following the second dose there was only a transient increase in plasma pepsinogen levels. After the animals were moved to pasture the mean levels of plasma pepsinogen of both groups oscillated from 600 to 1,900 mU in the vaccinates and from 400 to 2,200 mU in the controls up to October. From October onwards the level of the controls were considerably higher and reached a level of 3,500 mU, which was maintained until the last week of the experiment when the animals were housed.

Parasitological Data

Faecal egg and larval counts

The mean trichostrongyle egg counts of both groups are plotted in Fig. 6. Individual counts are presented in Appendix B, Table 13. The faeces of all the calves remained negative for nematode eggs or D. viviparus larvae during the vaccination period. After moving to the experimental paddocks, they commenced excreting trichostrongyle eggs just after the third week. In

Figure 5. Mean plasma pepsinogen levels of 2 groups of calves which grazed on *O. ostertagi* contaminated pasture during 1974. Group 1 was vaccinated with 2 x 100,000 γ-irradiated *O. ostertagi* L_3 and 2 x Dictol prior to grazing; Group 1 received only 2 x Dictol.

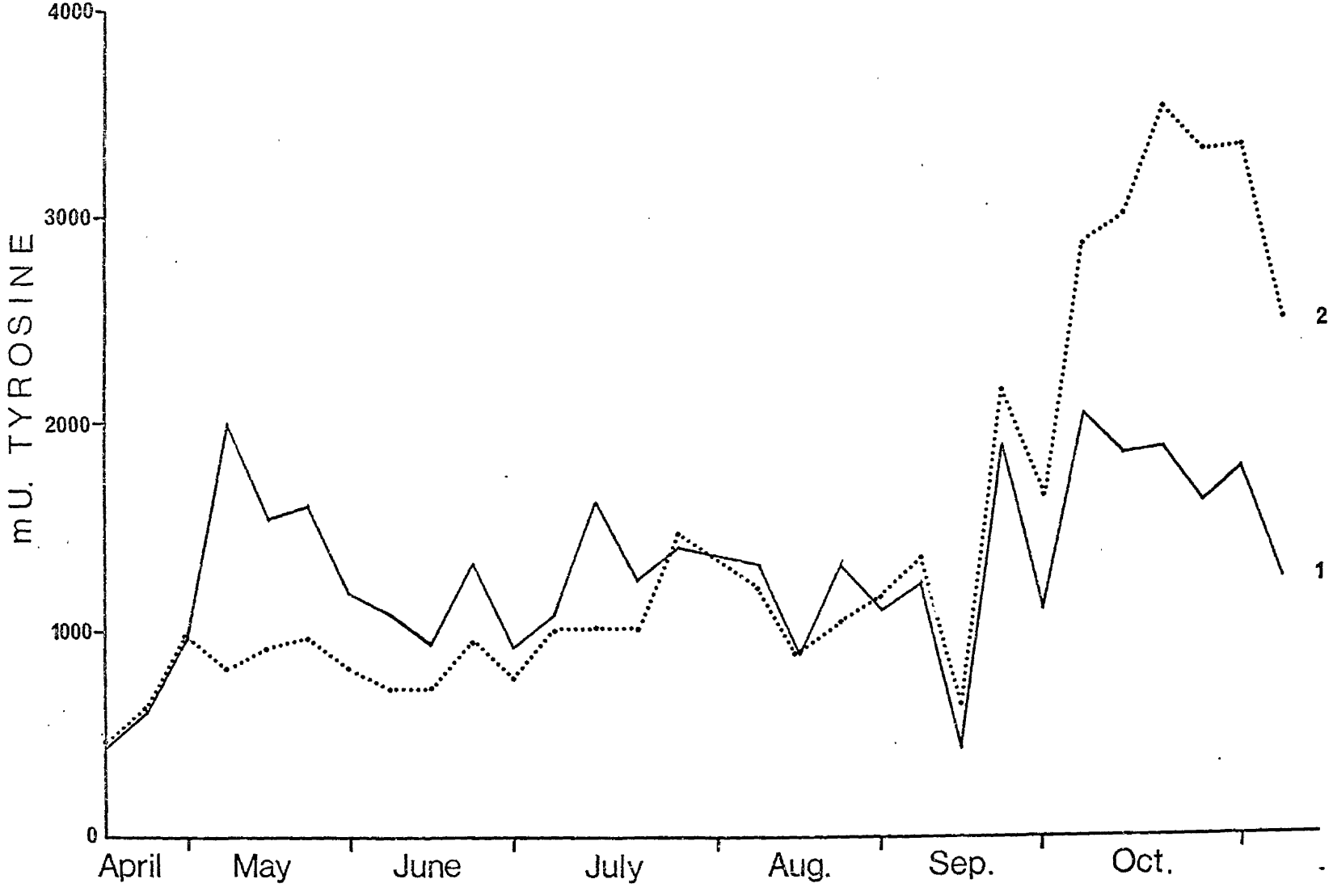
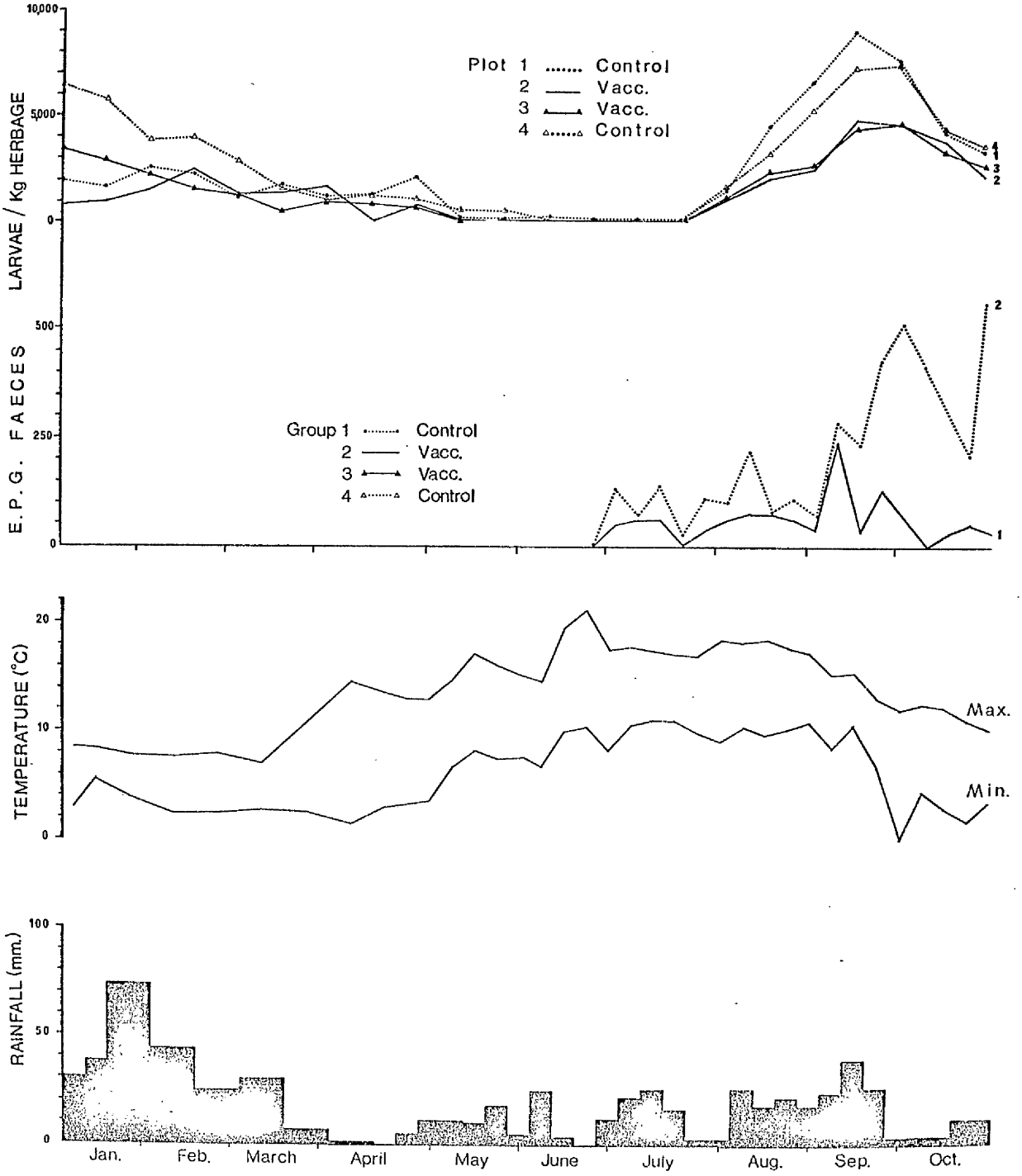


Figure 6 Details of temperature and rainfall in 1974, pasture larval counts of O. ostertagi L_3 from 4 plots grazed by separate groups of calves and mean trichostrongyle faecal epg. counts from these calves (Group 1 was vaccinated against O. ostertagi and D. viviparus; Group 2 only against D. viviparus).



general, the egg output was lower in the vaccinates. In the latter, the peak egg production was reached in the middle of September, when the mean was 240 and then decreased, while the controls were still eliminating a mean of 562 epg at the end of the trial.

Only a few Nematodirus spp and Strongyloides papillosus eggs, D. viviparus larvae and Eimeria spp oocysts were found during this trial. Data pertaining to these species are shown in Appendix B, Table 14.

Worm recoveries

The individual numbers of gastrointestinal worms and D. viviparus recovered from the calves are presented in Table 5. The same parameters for classification as in the first experiment were used for O. ostertagi in this year. There was no statistically significant difference in the numbers of worms present at post-mortem of the calves of both groups. Worm burdens of other gastrointestinal nematodes present are given in Appendix B, Table 15.

Pasture larval counts

As explained before, from July onwards the vaccinated group grazed alternatively (every other week) on paddocks 2 and 3 and the controls on paddocks 1 and 4.

There was a gradual decline in the number of larvae recovered from the herbage from January 1974, until late spring (Fig. 6). When the calves were turned on the pasture in June, the numbers of larvae recovered were extremely low (a maximum of 135 L_3/kg) in paddock 1 and negative in the other three. This situation remained

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TABLE 5.

Gastro-intestinal and lungworm counts at post-mortem of 2 groups of calves grazed on naturally contaminated pastures during 1974; prior to grazing one group was immunised against Ostertagia ostertagi and Dictyocaulus viviparus with irradiated larvae and another group only against lungworm.

	Ostertagia ostertagi							
Calf Number	Total	Male	Female	Ratio M:F	Total L_4	% L_4	Other Trich.*	Lungworm
Vaccinates								
204	64,800	2,400	2,700	1:1.1	59,700	92.1	4,600	-
238	9,000	400	800	1:2.0	7,800	86.7	4,800	1
885**	2,700	1,400	1,300	1:0.9	-	-	7,500	613
887**	2,000	700	1,300	1:1.9	-	-	800	117
903	5,700	500	700	1:1.4	4,500	78.9	4,400	-
Mean	16,840				24,000	85.9		
S.E.	12,054				17,875	3.8		
Controls								
196	58,300	3,600	3,400	1:0.9	51,300	88.0	6,200	2
881	15,200	1,800	2,000	1:1.1	11,400	75.0	2,900	-
888	26,600	4,600	4,500	1:1.0	17,500	65.8	8,500	-
891	75,300	9,100	11,400	1:1.3	54,800	72.8	2,900	3
Mean	43,850				33,750	75.4		
S.E.	13,893				11,235	4.6		

* Include nematodes of the genera Trichostrongylus, Cooperia and Nematodirus.

** Died in early July.

until the second half of July, and thereafter the numbers of L_3 in all plots increased steadily until the middle of September. From October the number of larvae recovered from the 4 plots then declined steadily. In the last herbage sample, taken on 31st October, the counts ranged from 2,200 to 3,500 L_3/kg. The number of O. ostertagi L_3/kg are shown in Appendix B, Table 16.

It is interesting to note that eggs appeared in the faeces of most calves prior to significant numbers of larvae being detected on the herbage.

As in the year before, the herbage larvae were predominantly O. ostertagi, with a few N. helvetianus and C. oncophora being found.

Data on the later species are recorded in Appendix B, Table 17.

Lungworm larvae were not detected in any of the herbage samples examined during 1974, although larvae were present in the faeces of some animals and adult lungworms were present at post-mortem of the calves.

Climatic Data

Climatic data from 1974 pertaining to the mean weekly maximum/minimum temperatures and mean weekly rainfall are also shown in Fig. 6 together with the trichostrongyle faecal egg counts and the herbage larval samples. The pattern of weather in 1974 was abnormal in that during the spring period the level of rainfall was particularly low, thus hardly any rain fell during the month of April. Thereafter,

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rain fell consistently during the year although it was never particularly heavy. The temperature figures were different from 1973 in that higher temperatures were recorded during the late autumn and early winter period.

Post-mortem Data

The pH of the abomasal contents of the calves at post-mortem are shown in Table 6. The 3 vaccinates from the first post-mortem was obtained had a mean of 2.9 while in the controls the mean was slightly higher reaching 3.8. Only one calf No. 891 had a level which could be considered abnormal being 4.5.

The lesions present at post-mortem of the calves this year were similar to those in the calves slaughtered in 1974 except that they were markedly less severe and the nodular lesion was more distinct with coalescence and oedema being less marked.

TABLE 6.

pH of the abomasal contents at post-mortem of calves grazed on Ostertagia contaminated pastures during 1974; prior to grazing some calves (vaccinates) were immunised with γ-irradiated O. ostertagi and D. viviparus larvae. The controls were immunised against D. viviparus only.

Group	Calf Number	pH
Vaccinates		
	204	3.5
	238	2.6
	Mean	2.9
Controls		
	196	3.6
	881	3.5
	888	3.7
	891	4.5
	Mean	3.8

The purpose of attenuating nematode larvae is to stimulate a sufficient degree of immunity when administered to the appropriate host without causing any significant pathogenic effect. With O. ostertagi it has been previously shown[36] that the vast majority of larvae attenuated by X-rays at 60 Kr will fail to reach the egg-laying adult stage but develop to the later larval stages which cause only minor damage to the abomasal mucosa; the latter was monitored by plasma pepsinogen levels, acknowledged as a good indicator of mucosal damage[14]. In the first of the current experiments the attenuation using γ-rays at 60 Kr was not completely successful in that 50 epg appeared in the faeces of 2 vaccinated calves following vaccination while the plasma pepsinogen levels rose to a moderate level (from <1,000 mU to ca 2,000 mU) after each vaccination, only to fall again almost to pre-vaccination levels. In the second experiment, also using γ-rays eggs were not detected in the faeces of calves following vaccination, and the plasma pepsinogen levels did not increase to the level expected following the second vaccination. No obvious explanation was obtained to account for the disparity in these results, hence two irradiation attempts.

The aim of the current experiments, was a) to study the protection conferred against the natural challenge by two immunising doses of γ- irradiated O. ostertagi

larvae, and b) to study the effect of introducing calves vaccinated with γ-irradiated O. ostertagi larvae on successive years on pasture levels of O. ostertagi.

From the results obtained in the first year, i.e. 1973, it is clear that no significant protection against natural challenge was achieved by double vaccinating calves with attenuated larvae. Thus, the mean O. ostertagi worm burdens of two groups of 3 vaccinated calves (2 of which died) were 68,800 and 145,067, whereas the mean burdens of 2 groups of control calves were 136,467 and 158,800. In previous studies[36] in the immunisation of calves with X-irradiated O. ostertagi larvae prior to field challenge the immunised and non-immunised control calves grazed on the same field, a policy which may have unfairly increased the challenge available to the immunised calves due to cycling of the infection by the controls. Nevertheless, in one of these trials 2 out of 5 immunised calves were highly immune as judged by the worm burden at post-mortem. In the current studies during 1973, none of the vaccinated calves could be considered to have developed a significant degree of immunity as reflected by their post-mortem worm counts.

Possibly the level of pasture infection in 1973 created by the "seeder" calves was too high (between 6-18,000 L_3/Kg) and overwhelmed any resistance produced as a result of vaccination. It was hoped that in the second year, i.e. 1974, this situation would not arise

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as the initial challenge would come from surviving overwintered L_3. Unfortunately, in 1974 the number of calves under experiment was reduced due to an outbreak of mucosal disease; nevertheless, 2 of the Ostertagia vaccinated calves had extremely low burdens at post-mortem in November, e.g. (5,700 and 9,000) when compared with the controls, although the mean group worm burdens were not significantly different. This result, in fact, was similar to that obtained by Armour in his studies[36], and may be related to the lower level of challenge experienced by the calves in 1974. A direct comparison cannot be made with Armour's experiments, since he did not measure the level of pasture infectivity.

Although lower pasture levels of O. ostertagia L_3 were present in the second year, this cannot be ascribed to any reduction in the biotic potential of the worms established in the vaccinated calves during 1973 for two reasons, 1) the faecal egg counts of both vaccinated and control calves increased during 1973 (see Fig. 3) and, 2) the level of infection on all of the paddocks was similar early in 1974 (see Fig. 6). A more rational explanation is that the mortality of the surviving overwintered L_3, known to occur annually[28] was particularly high in the spring of 1974 due to the excessive dry weather (see Fig. 3); this factor coupled with the later introduction of the calves on to the pasture in 1974,

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i.e. June, resulted in only negligible numbers of L_3 being initially available to challenge the calves. However, a definite modifying effect of the Ostertagia vaccine was apparent in that the faecal egg counts of the vaccinates were lower than those of the controls, and also the level of Ostertagia L_3 which developed on the plots grazed by the vaccinates was lower than those which built up on those grazed by the controls. Thus, the mean maximum faecal egg count of the vaccinates was 240 whereas that of the controls was 562. The mean maximum count of Ostertagia larvae on the plots grazed by the vaccinates were 4,769 and 4,596 whereas those on the control plots were 7,364 and 8,910.

In summary, therefore, it would appear that immunisation of calves with irradiated O. ostertagi larvae can have a significant effect on thc burdens subsequently established, and indirectly affect the level of future pasture infections with the infective stages of the parasite. The success of such immunisation is however dependent on the level of challenge to which the immune calves are initially exposed and if the challenge is high the host's immunity may be readily overcome.

Several other interesting points emerge from the results pertaining to the results of infection with O. ostertagi and other trichostrongyles.

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First, the increase of the pasture level of infection occurred in both years after mid-July; this result agrees with previous epidemiological studies on ostertagiasis using either pasture counts or tracer calves[28,51]. However, in contrast to the results obtained by Michel[28] the high pasture levels attained during the summer were not maintained throughout the late autumn and early winter, and in the current experiment fell rapidly during late autumn, (see Figs. 3 and 6). Whether this fall is due to a genuine reduction in numbers of larvae due to their mortality, or is merely a reflection of decreased motility of the L_3 and therefore of their availability on the herbage, remains to be proved.

Secondly, the marked increase in numbers of inhibited or arrested L_4 stages found in calves during the late autumn[27,51] was again noticed; thus, the calves which died during the summer or early autumn (Tables 2 and 5) had very low populations of inhibited L_4 or were negative compared to those slaughtered later in the season. As in previous studies[52] there was no correlation between the proportion of adults and the numbers of inhibited L_4.

Thirdly, it is interesting that, particularly in the first year, the liveweight gains of the control calves were superior to those of the vaccinates. While this may have been due to the control calves being initially heavier, the fact that the controls maintained a better weight gain performance during the grazing

season than the vaccinates suggests that some other factor or factors are operating. In this context it may be significant that animals given repeated infections[47] suffered more severe abomasal damage than animals given a large single infection[7]. Australian workers have noted a particularly severe ostertagiasis when adult animals, previously exposed to the parasite, were then subjected to natural challenge; these authors postulated a hypersensitivity reaction was responsible for the exaggerated mucosal response . Although theoretically attractive this argument is not borne out by the plasma pepsinogen levels of the current experiment in which a similar degree of damage was noted in vaccinated and control animals after challenge.

Fourthly, the presence of Cooperia oncophora and Nemotodirus helvetianus, presumably originating from the "seeder" calf obtained from the Medicine Clinic at the Veterinary School; this calf was suffering from naturally induced parasitic gastroenteritis and harboured nematodes from several different genera. Apart from one or two occasions during the 2 year period of observation the herbage levels of C. oncophora and N. helvetianus remained low, the larval infections being dominated by O. ostertagi as is commonly seen in South West Scotland[36]. The N. battus infections presumably originated from the sheep which had grazed on the pasture accidentally.

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Finally, and perhaps the most interesting aspect of the entire experiment, was the occurrence of clinical parasitic bronchitis in the Dictol immunised calves. In both years these cases occurred in the control calves vaccinated with Dictol alone or the vaccinates given the combined vaccination with Dictol and O. ostertagi attenuated larvae. Thus, in 1973, 4 out of 6 control calves had lungworm burdens ranging from 4 to 253 and in 1974, 4 out of 5 control calves had lungworm burdens ranging from 2 to 3. In 1973, of the calves given both parasitic vaccines, 5 out of 6 harboured D. viviparus adults at post-mortem, the burdens ranging from 3 to 448; in 1974, in the calves given the two vaccines D. viviparus were present in 4 out of 5 and ranged from 1 to 613.

It is particularly puzzling as to where the pasture infection with D. viviparus could have originated in 1973. Both paddocks had not been grazed for 2 years by other cattle and the "seeder" calves were not infected with lungworm when examined at post-mortem. The faeces of all the calves were negative following vaccination for D. viviparus larvae (or at the most contained less than 50 larvae per gram since in the technique used the finding of one larva represents 50 per gram) until September 12th when larvae appeared simultaneously in calves given Dictol vaccination alone and in those given Ostertagia and Dictol vaccines; this occurred 1-2 weeks after lungworm larvae were first detected on the herbage (see Fig. 3).

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From the data available it is difficult to even speculate on the source of infection but it serves to emphasise the existing gap in our knowledge on the epidemiology of parasitic bronchitis. The recent suggestion by Kaarma[54] (1969) that earthworms may act as reservoir hosts for D. viviparus larvae is a most interesting one and worthy of further investigation; certainly, the proven existence of a reservoir host for lungworm would help to clarify some of the unexplained outbreaks of parasitic bronchitis.

Since the Ostertagia vaccinated calves appeared more susceptible to lungworm than the controls, it is possible that the antigenic competition created by the simultaneous vaccination of these calves against the two parasites may have reduced the immunity engendered by Dictol. Alternatively, the onset of clinical ostertagiasis in July and August prior to the outbreak of parasitic bronchitis may have resulted in a suppression of the existing Dictol-induced immunity.

Similarly, in 1974, lungworms were present in both groups of calves, but again the numbers present were higher in those given both parasitic vaccines. In this year, apart from the effect of ostertagiasis, the possible role of the virus of mucosal disease in suppressing the Dictol-induced immunity must also be considered.

Although lungworm larvae were recovered from the faeces of the calves and adult worms from the lungs at post-mortem, D. viviparus larvae were not detected on

herbage examination. This lack of sensitivity on the pasture, capable of initiating clinical disease, exposes the limitation of herbage sampling as a monitoring process for predicting parasitic bronchitis; this is in contrast to the results obtained with similar techniques which can successfully monitor trichostrongyle larval populations on pasture.

SUMMARY

In 1973 and 1974 attempts were made to immunise 10 week-old parasite-naive calves against the stomach worm Ostertagia ostertagi by administering 2 oral doses of 100,000 X-irradiated infective larvae (L_3) at an interval of 4 weeks.

In both years 2 replicate groups of 3 calves immunised with irradiated O. ostertagi L_3 and 2 replicate control groups grazed from May through October on four separate paddocks each of 0.3 hectares, which had been naturally contaminated with O. ostertagi. Prior to grazing all the calves were immunised against the lungworm Dictyocaulus viviparus with the proven irradiated larval vaccine, Dictol.

In 1973, the pasture levels of O. ostertagi L_3 were high in the spring and again after mid-summer, and clinical ostertagiasis occurred in both the immunised calves and the controls. At post-mortem there were no significant differences in the magnitude of the O. ostertagi burdens present.

In 1974, the pasture levels of O. ostertagi were low in the spring but increased from mid-summer, but did not reach the high levels of 1973. Although the numbers of calves in each group were unfortunately reduced due to a severe outbreak of mucosal disease, there was sufficient evidence to indicate a positive effect of immunising procedure in reducing the level of O. ostertagi eggs in

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the faeces, L_3 on the herbage and worm burdens at post-mortem. It appears that the successful outcome of immunisation against O. ostertagi is dependent on the level of challenge.

In both years, some of the calves suffering from clinical ostertagiasis subsequently developed parasitic bronchitis, and at post-mortem had considerable numbers of lungworms present. It is postulated that prior experience of the calves to ostertagiasis (and in 1974 mucosal disease) may have suppressed the immunity induced by Dictol.

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SECTION 3.

A COMPARISON OF TWO TECHNIQUES FOR THE RECOVERY OF *OSTERTAGIA OSTERTAGI* AND *DICTYOCAULUS VIVIPARUS* LARVAE FROM HERBAGE

INTRODUCTION

During 1973 an adaptation of the technique developed by Smeal and Hendy[1] for the detection of nematode larvae on the herbage was used. Although O. ostertagi, Cooperia oncophora, Nematodirus spp. and D. viviparus were readily recovered it seemed pertinent to compare during 1974 the effectiveness of this technique with that traditionally used in Britain and developed at the Ministry of Agriculture Laboratories at Weybridge[2].

The two techniques are outlined in the Materials and Methods, page 26. Basically, the principal difference is that in the adapted method of Smeal and Hendy there is no filtration of the larvae through milk filter pads and no use of a Baermann apparatus; in contrast the Weybridge technique utilises both of these procedures. It was hoped that by adapting a technique such as Smeal and Hendy's the detection of small numbers of larvae, especially sluggish larvae such as D. viviparus, would be improved without the trapping effect created by filtration.

EXPERIMENTAL DESIGN

Herbage samples were taken in May and July 1974 from the 4 plots and were then processed using both techniques; theoretically the numbers of larvae on the pasture during these months would be low. Further samples taken from August to September, i.e. when herbage population are usually high and so these techniques could be compared

under conditions of both low and high levels of herbage larval infection.

RESULTS

The numbers of O. ostertagia L_3 recovered from the 4 plots during May through September are shown in Table 1. D. viviparus L_3 were not recovered from any of the herbage samples and these negative results have not been included in the table. Only low numbers of O. ostertagi L_3 were recovered from May 15th until July 24th.inclusive and these results provide data for comparison of the techniques in lightly contaminated herbage. From August 7th until September 18th inclusive, large numbers of L_3 were present and these results provide data for comparison of the techniques in heavily contaminated herbage.

DISCUSSION

The results show that where low numbers of O. ostertagi L_3 were present, i.e. less than 100 per kg the Weybridge technique proved to be more sensitive than the Smeal and Hendy[1] technique in 7 out of 17 occasions; in the remaining 10 samples with less than 100 L_3 per kg there were only minimal or nil differences in the numbers of L_3 recovered by the two techniques; when over 100 L_3 per kg or more were present there was little to choose between the two techniques; in some occasions e.g. on 15th May in Plot 4

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TABLE 1. The numbers of Ostertagia ostertagi infective larvae recovered from herbage samples from May through September 1974, using two different techniques.

Date	Plot 1		Plot 2		Plot 3		Plot 4		S & H	W
	S & H	W	S & H	W	S & H	W	S & H	W	Mean ± S.E.	Mean ± S.E.
15/5	128	453	0	526	120	120	532	93	195 ± 116	298 ± 112
25/5	0	69	0	0	0	39	458	17	114 ± 114	31 ± 15
12/6	135	34	0	0	0	19	0	29	34 ± 34	20 ± 8
26/6	0	52	0	13	0	0	0	23	0 ± 0	22 ± 11
10/7	19	24	0	0	0	0	11	14	7 ± 5	9 ± 6
24/7	0	130	0	0	0	0	50	190	12 ± 12	80 ± 48
7/8	1412	1751	1006	967	1029	1543	2575	2392	1505 ± 368	1663 ± 294
21/8	4372	5643	2019	1741	2279	2361	3216	3741	2971 ± 533	3371 ± 865
4/9	6461	7326	2466	2136	2673	2981	5184	5496	4196 ± 975	4485 ± 1186
18/9	8910	9174	4769	4468	4433	4250	7218	7638	6332 ± 1059	6382 ± 1210

S & H Smeal and Hendy's technique.

W Weybridge technique.

the Smeal and Hendy's technique proved more sensitive whereas on the 24th July the Weybridge technique was better.

During August and September 1974, when large numbers of L_3 were recovered from the herbage, the numbers recovered by each technique were reasonably similar and the differences were clearly not significant.

In retrospect it might have been marginally better to adopt the Weybridge technique, as the standard for the field experiments, but the accuracy of Smeal and Hendy's techniques has been claimed as 90%[1] and was therefore adopted as the standard for the experiment.

Furthermore, by eliminating the process of filtration of larvae through sand in the Smeal and Hendy's techniques the recovery should have been better, particularly as we examined 20% of the sample and not 10% as described by Smeal. Also, since D. viviparus L_3 are notoriously labile when subjected to filtration or sieving and the Weybridge technique is reported to recover only 40%[2] of these larvae, it was decided to use the Smeal and Hendy technique. In 1973, D. viviparus L_3 were recovered from the herbage by the latter method, but in 1974 they were not present in any of the samples examined by either method; the absence of D. viviparus L_3 from these herbage samples is difficult to explain, since clinical parasitic bronchitis occurred in calves grazing the areas which were sampled and 613 lungworms were recovered from one animal.

SUMMARY

Two techniques for pasture larval recovery were compared. The Weybridge method required sieving and filtration of pasture sample washings, while the method developed by Smeal and Hendy involved repeated sedimentation of these washings. In late autumn and early summer, when the larval population on pasture was below 100 L_3/Kg of grass, in general the Weybridge method proved to be more sensitive. From late summer, when the numbers of larvae increased markedly, the recovery by both methods were similar.

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SECTION 4.

STUDIES ON VACCINATION OF SHEEP AGAINST *HAEMONCHUS CONTORTUS*.

INTRODUCTION

Haemonchus contortus (Rudolphi, 1803) Cobb, 1898 the 'barber's pole worm' or 'bankrupt worm' is a frequent parasite of the abomasum of sheep and goats throughout the world although it is of particular economic importance in tropical and sub-tropical areas.

This nematode was also thought to be a common parasite of cattle, but work by Roberts, Turner and McKevett[1] has established that the species in cattle is usually H. placei and not H. contortus. This species differentiation is based on certain morphological characters e.g. in the male worm the mean length of the spicules and the distance between the hooks and the tips of the spicules and in the female the length of the vulvar flap; since several workers have recorded forms of Haemonchus with a morphology intermediate between that of H. contortus and H. placei it seems reasonable to assume that interbreeding of the two species occurs under natural conditions[2].

The life cycle of H. contortus is, like other trichostrongyles, direct and may be summarised as follows: Eggs laid by the fertilised female in the abomasum are voided in the faeces; the number of eggs passed is usually extremely numerous, since the parasite has a high biotic potential and each female worm is capable of producing 5,000 to 10,000 eggs per day[3,4,5].

Development from the egg through the L_1 and L_2 to the infective L_3 takes place in the faecal pellet, and significant numbers of L_3 develop when the mean monthly rainfall exceeds 53 mm and the mean minimum temperature reaches at least 18°C[4,6,7,8,9,10,11]; as the temperature reaches to a maximum of 30°C the period of development shortens to 3 to 4 days[7,8,10].

Studies by Ellenby[12], Crofton[13] and Waller and Donald[14] have demonstrated that only the L_3 stage of H. contortus is resistant to prolonged dessication, and since the parasite is particularly prevalent in tropical areas, knowledge on the longevity of this stage under arid conditions is of tremendous importance. Recently, Allonby[15] has shown in Kenya that a small but significant number of L_3 are capable of survival on pasture for as long as 6 to 12 months, despite a prolonged period of aridity. The L_3 is also capable of withstanding low temperatures and a proportion can survive the winter in southern England[16].

Once ingested the L_3 exsheath in the rumen and moult in the abomasum to become the L_4 about 2 days later[8,17]; they penetrate the abomasal mucosa between the gastric glands but seldom fully enter the latter[8,17]. Growth of the parasite may be arrested at this stage for lengthy periods, either due to a previously experienced stimulus while free-living on the pasture[18,19,20,21,22,23,24], or as a result of acquired immunity[25,26,27,28]. The developing L_4 emerges from the mucosa by day 6, grows and

moults to become L_5 at about the 10th day after ingestion and reaches the mature adult stage on day 15. The adults measure just over 2.5 cm in length and are red in colour from the ingestion of blood. Blood loss attributable to the haematophagic activity of the parasite begins 6-12 days after ingestion[29,30,31]. This increases markedly when the adult stage is reached and it is calculated that each adult worm can remove 0.02-0.07 ml of blood per day[29,31]; in heavy infections the daily blood loss may therefore reach 300 ml[32].

In addition to producing an anaemic state the developing stages of the parasite, if present in large numbers cause an increase in the pH of the abomasal content, from the normal 2.5 to 6 or 7, a change which clearly impairs digestion[17,33].

Perhaps the most interesting aspect of the whole host/parasite relationship with H. contortus is the rather unusual reaction of the host to the parasite in that there is little evidence of the development of a useful degree of acquired immunity against H. contortus in sheep and goats under normal grazing management. In tropical and sub-tropical areas sheep and goats become infected at an early age and are often continually and successfully infected throughout life[4,34,35].

The survival of sheep which are set-stocked on permanent pasture and not subjected to regular and frequent anthelmintic treatment is customarily attributed to the periodic expulsion of the entire adult worm burden[34].

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This reaction is termed the self-cure and was first introduced by Stoll[36] to describe a sudden and dramatic fall in faecal egg counts which occurred in two lambs subjected to continuous natural reinfection with H. contortus larvae from pasture. The epidemiological significance of the phenomenon was subsequently shown by Gordon[34] who demonstrated that self-cure occurred regularly and consistently in entire flocks of sheep grazing in H. contortus endemic areas in Australia. Gordon also observed that not only was self-cure associated with a dramatic fall in faecal egg counts, but was also associated with the expulsion of the adult H. contortus burdens of the affected sheep. The occurrence of self-cure was generally recorded once or twice yearly and invariably occurred shortly after the onset of a period of rainfall which led Gordon to suggest that the phenomenon was possibly attributable to an anthelmintic factor present in the growing pasture. In a later series of experiments however, Stewart[37,38,39] showed that a natural or experimental challenge with H. contortus larvae frequently produced a similar fall in faecal egg count and expulsion of a pre-existing adult infection. From these observations Stewart[39] concluded that the mechanism of self-cure depended on an immediate type hypersensitivity reaction in the abomasal mucosa created by the antigenic stimulus of the newly acquired larvae. It has been subsequently assumed that self-cure is a

flock phenomenon dependent on a similar mechanism and that the significance of the rainfall was that a large number of infective larvae became available to sheep.

There are however certain features of a naturally occurring flock self-cure which are inconsistent with an immunological explanation. Thus, self-cure occurs at exactly the same point in time in mature ewes and young lambs, is expressed equally in sheep with high or low adult worm burdens and is not usually followed by effective resistance to reinfection[34,35,40]. More recent work by Allonby and Urquhart[41] in East Africa has shown that in Merino sheep the onset of self-cure was found, as judged by a dramatic fall in faecal egg counts, to be simultaneous in sheep grazing on infected pasture and in sheep grazing on parasite-free pasture. Furthermore, the results of post-mortem examinations carried out before and after self-cure showed that a marked and equal loss of adult worm burdens had also occurred under both grazing systems. These authors therefore postulated that self-cure of H. contortus infections under natural conditions occurs in the absence of reinfection and is apparently not necessarily immunological in origin.

Another feature to emerge from Allonby's study[15] was that sheep of haemoglobin type A (HbA) displayed self-cure more frequently than sheep of haemoglobin typeAB or B (HbAB or HbB). This interesting finding

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supported the suggestion of Radhakrishnan, Bradley and Loggins[42] that Hb type may influence the host/parasite relationship. In their studies, Florida Native sheep of HbA were generally more resistant to the development of an experimental H. contortus infection than those of the HbAB type.

Despite the apparent lack of acquired immunity in the field, considerable research effort has been directed towards a study of acquired immunity to H. contortus in sheep under experimental conditions. These studies have been carried out on various breeds of sheep using both normal and X-irradiated larvae and also strains of H. contortus taken from wild ruminants.

The first attempt to immunise sheep against H. contortus with normal larvae was made by Stoll[43], who injected L_3 by the subcutaneous or intraperitoneal route and found that these larvae subsequently protected sheep against an oral challenge which was sufficient to kill the previously uninfected controls. Later Stoll[44] repeated this work and found that 9 out of 10 sheep of an undescribed breed were highly resistant when they were immunised previously with large number of exsheathed L_3 given either subcutaneously or intraperitoneally; however, Soulsby and Stewart[45] and Wilson and Samson[46] were unable to confirm these results.

The first fully documented experimental attempt to immunise sheep with normal H. contortus L_3 given by the conventional oral route was made by Manton, Peacock,

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Poynter, Silverman and Terry in 1962[47]. These workers gave 9,000 L_3 either in 2 equal doses or as a trickle infection over a period of 60 days to Dorset Down lambs aged 10-12 months old. These lambs recovered from the infection and completely resisted a challenge with 15,000 L_3 one month later. In contrast when Dorset Horn lambs aged 2-4 months were given a similar immunising schedule using 3,000 L_3 they were as susceptible to subsequent challenge with 5,000 L_3 as the parasite-naive controls. These experiments emphasise the relationship of the age of the sheep and its ability to mount an effective immune response to H. contortus. Further studies by Brambell, Charleston and Tothill[30] confirmed the ability of older lambs (9 month-old Greyface x Scottish Blackface) to acquire an immunity following the administration of normal larvae.

The possible influence of breed or strain of sheep on acquired resistance to H. contortus is apparent from the contrasting results of Conway[48] and Dineen, Donald, Wagland and Offner[26]. In Conway's experiments, sheep of an undescribed breed aged 3 to 18 months, failed to develop any significant resistance following either single or serial infection with normal H. contortus L_3. On the other hand Dineen and his colleagues using Merino lambs aged 2-3½ months demonstrated that while the administration of a large single dose of 3,000 larvae resulted in severe haemonchosis, lambs receiving the same total number of larvae in 30 daily doses of 100

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developed only minor clinical signs. At post-mortem of the lambs given the serial doses of larvae a considerable proportion of the worms were arrested at the L_4 stage and the authors considered this to be due to the immune response of the host.

Apart from the influence of the breed of sheep on the host/parasite relationship with H. contortus it has been demonstrated by the Cornell workers[49,50,51] that within breeds, certain strains are more resistant to H. contortus and this can be genetically transmitted. In their studies it was shown that the progeny of one ram in a flock were highly resistant to H. contortus compared with the progeny of other rams.

The effect of the size of immunising infection was studied by Christie and Brambell[52] who produced a significant immunity to subsequent challenge in 9½ week-old Scottish Blackface lambs by using a massive immunising dose of 6 daily doses of 25,000 L_3 followed by anthelmintic therapy with thiabendazole, a further 8 daily doses of 25,000 L_3, and yet another anthelmintic treatment.

In an attempt to study the stage of the life cycle associated with the development of resistance, Christie, Brambell and Charleston[53] immunised sixteen 7½ months old Scottish Blackface sheep with 2 immunising daily doses of 10,000 L_3 for a period of 10 days, and then removed all of the larval stages present with thiabendazole. On subsequent challenge 11 days later, there was evidence of a good immunity compared with parasite-naive controls,

presumably produced by the immunising larval infection. Later Wagland and Dineen[54] demonstrated that in Merino-Border Leicester cross breed lambs aged 3-4 months, when experimentally infected with H. contortus and then treated with an anthelmintic, there was an immunologically latent period of 2 weeks. After this there was an increase and then a fall in the resistance to challenge; the highest resistance occurred between 4 and 8 weeks after the initial inoculation of larvae. By 16 weeks after the anthelmintic treatment, the response to challenge was similar to that of parasite-naive controls.

The possible use of heterologous strains of H. contortus as immunising agents was investigated by Allen, Samson and Wilson[55]. In their study a strain from the pronghorn antelope was less pathogenic than a homologous strain and proved as immunogenic as the latter.

Attempts to immunise sheep against H. contortus using attenuated larvae were made by many workers following the successful immunisation of calves with X-irradiated Dictyocaulus viviparus larvae. Thus, Jarrett and his colleagues[56,57] immunised 7-8 months old worm-free Scottish Blackface lambs with either a single dose of 10,000 L_3 X-irradiated at 40 Kr or 2 doses at a month's interval; on a subsequent challenge one month later with 8,000-50,000 normal L_3 the number of worms recovered was markedly reduced compared with controls.

Bitakaramire[58] using a similar immunising schedule as Jarrett and his colleagues, and in 10 month-old Scottish Blackface sheep, reported a high resistance to subsequent challenge compared with non-immunised controls; he also demonstrated that the immune response to the challenge infection was directed against the L_3 and L_4 stages. Urquhart et al.,[59] also reported successful vaccination of Scottish Blackface sheep aged 17 months using two doses of 10,000 X-irradiated H. contortus L_3; however, the same workers were unsuccessful in their attempts to immunise Scottish Blackface lambs aged 5-12 weeks old using a similar immunising schedule[59,60]. The use of adjuvants, a reduction in the numbers of irradiated larvae in the immunising infection or fractionation of the challenge dose in no way improved the immunity in these young lambs.

Later, and using a different breed of sheep, Mulligan, Gordon, Stewart and Wagland[61] double-vaccinated 7 months old Merino lambs in Australia with a first dose of 2,000 and a second dose of 5,000 L_3, X-irradiated on this occasion at 60 Kr; 6 of the sheep were solidly resistant to subsequent challenge of 10,000 normal L_3 but the remaining 4 had worm populations ranging from 1,171 to 3,360. Further studies by Lopez and Urquhart[35] in Kenya with adult Merino sheep reared in an endemic area revealed an absence of immunity to challenge with

normal larvae following double-vaccination with 10,000 X-irradiated L_3. These workers postulated that exposure to infection with H. contortus in early life had the effect of interfering with the subsequent immune response of the adult host, since Merino sheep reared free from infection showed good immunity after vaccination between 7 and 24 months of age.

Clearly, from the contrasting results available in the literature, several points require elucidation. In particular the influence of breed of sheep, haemoglobin type, the innate immunological unresponsiveness of young lambs, the possible existence of an acquired immunological unresponsiveness and the effect of anthelmintic therapy on the acquisition of immunity, are worthy of further study.

The object of the experiments reported in this section was to investigate four of these points. First, to attempt to overcome the poor immunological response of young lambs by increasing the amount of antigen available, as suggested by the work of Christie and Brambell[52]; secondly, to attempt to confirm the theory of Lopez and Urquhart[35] that exposure in neo-natal life to H. contortus larvae produced a state of immunological unresponsiveness and so interefered in later life with the response to vaccination; thirdly, to study the influence of anthelmintic treatment on the ability of sheep to respond immunologically to H. contortus

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infections in later life; finally, to assess the role of haemoglobin type in the ability of sheep to respond to immunisation with γ-irradiated H. contortus larvae.

EXPERIMENT 1

This experiment was carried out to study the relationship between the numbers of irradiated H. contortus larvae used to immunise 10 week-old Scottish Blackface lambs and the subsequent degree of resistance of the lambs to a subsequent challenge with normal larvae.

Experimental Design

Three groups of 7 parasite-naive lambs, housed under parasite-free conditions were immunised with two doses of 10,000 (Group 1), 100,000 (Group 2) and 1,000,000 (Group 3) H. contortus L_3 which had been γ-irradiated at 60 Kr; the doses were given at an interval of 4 weeks. These groups and a non-immunised control group of 7 lambs were then challenged with 10,000 normal L_3 4 weeks after the second immunising infection. Three weeks after each vaccination the anthelmintic thiabendazole was given at 110 mg/kg. The experimental design plus details of controls on the γ-irradiation and infectivity of the larvae used are given in Table 1.

Observations

The lambs were observed daily and given a thorough clinical examination at weekly intervals. Blood was collected also at weekly intervals and examined for packed cell volume percentages, haemoglobin concentration

TABLE 1

Design of Experiment 1.

Group	No. of Animals	Day 0		Day 28		Day 56	Day 86
1	7	10,000 γ-irradiated L_3	*	10,000 γ-irradiated L_3	*	10,000 Normal L_3	K
2	7	100,000 γ-irradiated L_3	*	100,000 γ-irradiated L_3	*	10,000 Normal L_3	K
3	7	1,000,000 γ-irradiated L_3	*	1,000,000 γ-irradiated L_3	*	10,000 Normal L_3	K
4	7					10,000 Normal L_3	K
5	1	10,000 γ-irradiated L_3		K			
6	1	10,000 Normal L_3		K			
7	1			10,000 γ-irradiated L_3		K	
8	1			10,000 Normal L_3		K	

* Thiabendazole one week before vaccination or challenge.

K day of slaughter.

and numbers of red blood cells present. At the same time the lambs were weighed and faecal samples collected from the rectum for estimation of the numbers of H. contortus eggs.

At post-mortem the abomasum was removed and the numbers of H. contortus adults and larval stages present in the abomasal contents and a digest of the mucosa were enumerated.

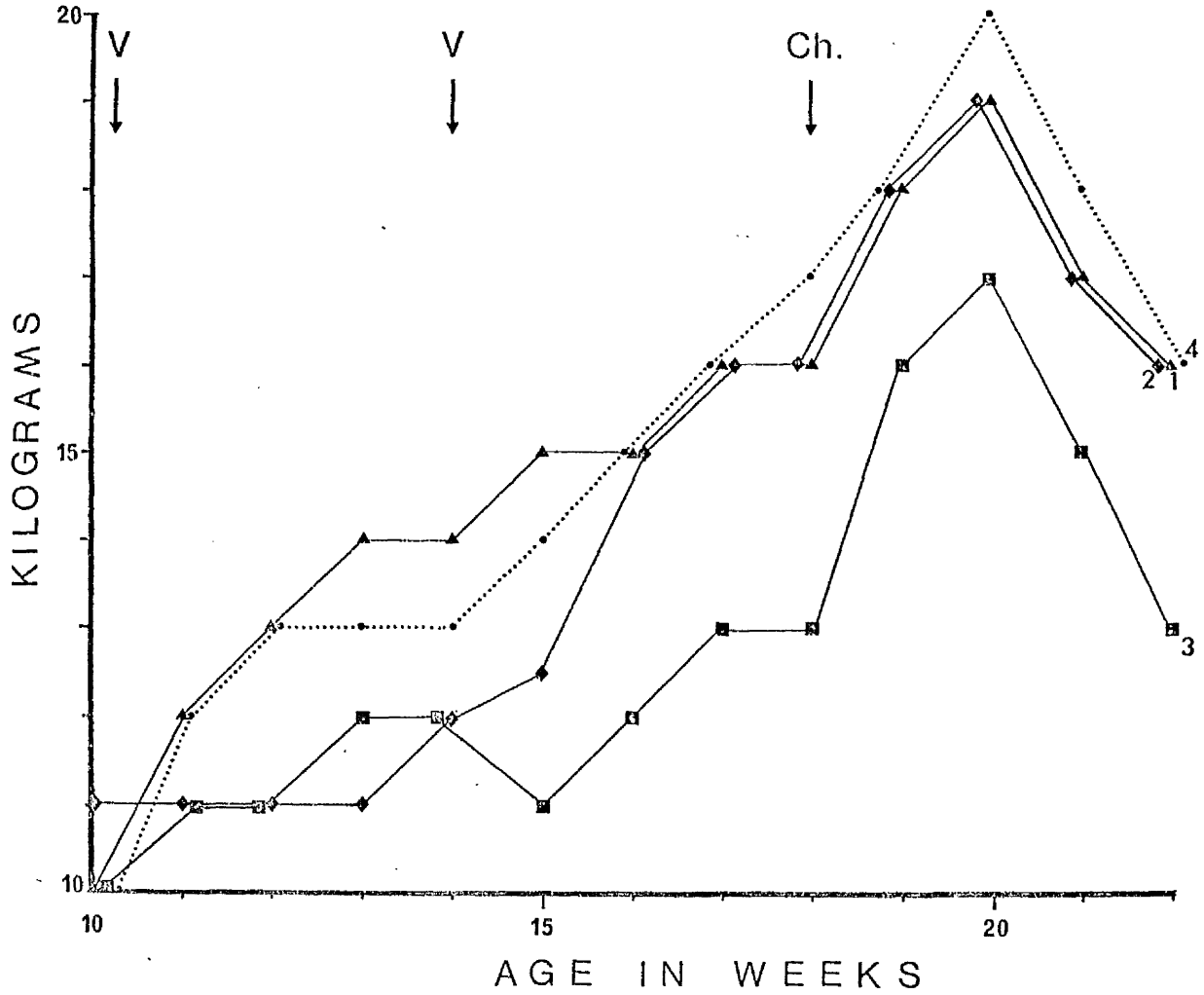
Results

Clinical Data

Two of the lambs of Group 3 died during the third week after receiving the initial dose of 1,000,000 γ-irradiated L_3 due to severe anaemia. The surviving animals of Groups 2 and 3 were listless and displayed pale mucous membranes and in certain instances an accelerated pulse. Following treatment with thiabendazole they recovered rapidly although after the second vaccination some of the lambs became ill again and 2 lambs of Group 2 and 1 lamb of Group 3 died. Following challenge another lamb of Group 3 died one week later.

The body weights of the lambs during the period of immunisation and after challenge are shown in Fig. 1 and individual weights are presented in Appendix C, Table 1. Following immunisation there was a reduction in weight gain of the lambs of Groups 2 and 3, although after the anthelmintic treatment those in Group 2 had

Figure 1 Mean body weights of 3 groups of lambs immunised (V) with 2 doses of 10,000 (Group 1); 2 x 100,000 (Group 2) and 2 x 1,000,000 (Group 3) γ-irradiated H. contortus L_3. These lambs and controls (Group 4) were challenged (Ch) 4 weeks after second vaccination.



recovered and were gaining weight steadily by the time of challenge. After challenge there was a severe loss of weight in the lambs of all 3 immunised groups and the controls, amounting in some cases to almost 50% of the live weight by the time of slaughter.

Haematological Estimations

Packed cell volume percentages (PCV)

The mean PCV's of the three groups of immunised lambs and their controls are shown in Fig. 2 and the individual values are given in Appendix C, Table 2. The mean PCV's of the vaccinated lambs decreased after the first immunising dose of irradiated larvae, the changes being most marked during the third week and affecting particularly the lambs given the higher immunising doses, e.g. the mean PCV of the lambs in Groups 2 and 3 were 14 and 19% respectively. Following the anthelmintic treatment the PCV levels increased and at the time of challenge had almost reached their pre-vaccination levels. After challenge with 10,000 normal L_3 the mean values of all the lamb groups decreased to between 19 and 24% during the third week; thereafter, the levels remained steady until slaughter one week later.

Haemoglobin concentration and red blood cell counts

The fluctuations in these parameters were similar to those of the packed cell volume percentages and to prevent unnecessary repetition have not been included in the results.

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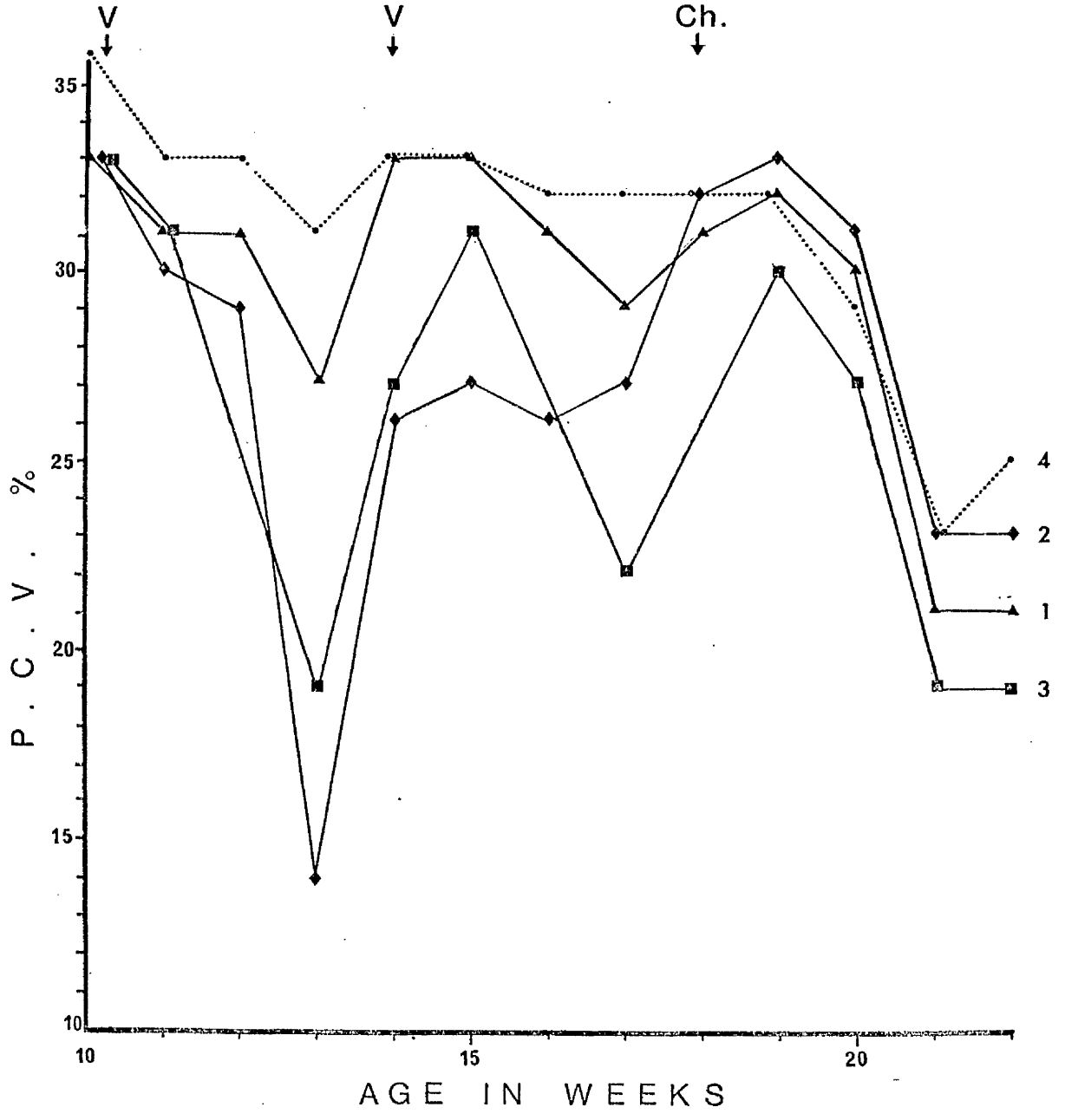
Figure 2 Mean PCV % of 3 groups of lambs immunised (V) with 2 doses of 10,000 (Group 1); 2 x 100,000 (Group 2) and 2 x 1,000,000 (Group 3) γ-irradiated H. contortus L_3. These lambs and controls (Group 4) were challenged (Ch) 4 weeks after second vaccination.



Parasitological Data

Faecal egg counts

Throughout the immunisation period the faeces of the lambs were negative for helminth ova. Three weeks after challenge H. contortus eggs appeared in the faeces of lambs in the three immunised groups and the controls and reached a peak during the 4th week when the faecal egg counts ranged from 24,000 to 42,000 eggs per gram. The individual H. contortus faecal egg counts of the lambs in Groups 1 through 4 are presented in Appendix C, Table 3.

Worm recoveries

The mean numbers and standard error of H. contortus present at post-mortem of lambs in the different groups are shown in Table 2 and the individual numbers of H. contortus in Appendix C, Table 4. All of the worms recovered were adults and arrested larvae were not found. There were no significant differences in the mean number of worms present in the lambs of the three immunised groups (Group 1 - 3,143; Group 2 - 2,760; Group 3 - 2,625) and the controls (Group 4 - 3,014).

The lambs used to control the effectiveness of the irradiation, namely Groups 5 and 7 had 0 and 200 worms respectively, while those inoculated to test the viability of the normal larvae, namely Groups 6 and 8 had worm burdens of 2,200 and 3,200 respectively.

TABLE 2

The mean numbers and standard error of Haemonchus contortus present at post-mortem of three groups of lambs immunised with irradiated larvae and subsequently challenged with normal larvae, together with control lambs.

Group	No. of Animals	Immunising Schedule with γ-irradiated Larvae Day 0	Day 28	Challenge with Normal Larvae Day 56	Mean Numbers of H. contortus at Post-Mortem on Day 86 Total Adults	Males	Females
1	7	10,000	10,000	10,000	3143±433	1900±346	1243±213
2	5	100,000	100,000	10,000	2760±287	1720±146	1040±160
3	4	1,000,000	1,000,000	10,000	2625±357	1475±335	1150±185
4	7	-	-	10,000	3014±243	1857±131	1157±143
5	1	10,000 γ-irradiated L_3	K		0	0	0
6	1	10,000 Normal L_3	K		2200	1100	1100
7	1		10,000 γ-irradiated L_3	K	200	100	100
8	1		10,000 Normal L_3	K	3200	1900	1300

N.B. All lambs received thiabendazole on days 21 and 49.
Larvae were irradiated at 60 Kr.

K = Killed.

EXPERIMENT 2

This experiment was designed to assess whether Scottish Blackface lambs exposed regularly to infection with H. contortus larvae from 10 weeks old would develop an impaired response to subsequent immunisation with γ-irradiated larvae when 9½ months of age.

Experimental Design

One to two thousand normal H. contortus larvae were administered orally to 7 parasite-naive Scottish Blackface lambs (Group 1) aged 10 weeks and the infection terminated 3 weeks later with thiabendazole given at 110 mg per Kg body weight. This procedure was repeated a 4-weekly intervals until the lambs were 38 weeks old. On each occasion one week elapsed between anthelmintic treatment and reinfection. At 38 weeks of age the 7 lambs of Group 1 and 7 parasite-naive sheep of the same age (Group 2) were immunised with 10,000 H. contortus larvae γ-irradiated at 60 Kr. Three weeks later thiabendazole was given to the 14 lambs and the immunisation procedure repeated one week after the anthelmintic; 3 weeks later another dose of thiabendazole was given. Four weeks after the second immunising dose the 14 immunised lambs and 7 parasite-naive controls of the same age (Group 3) were challenged with 10,000 normal H. contortus L_3. All the lambs were slaughtered 4 weeks later.

During the experimental period the lambs were housed under conditions known to prevent accidental infections

with nematode larvae.

The experimental design including the necessary irradiation and infectivity controls is shown in Table 3.

Observations

Apart from daily observation the lambs were weighed at weekly intervals when a more detailed clinical examination was made and blood and faecal samples collected for laboratory examinations. The latter consisted of PCV, Hb and RBC estimations on the blood and examinations of the faeces for the presence of parasite eggs.

At post-mortem the abomasum was removed and the H. contortus, recovered from the contents and digest of the mucosal wall, were counted. From each group the first 30 female worms were measured using a planometer and their lengths calculated in millimetres (mm).

Results

Clinical Data

One lamb from Group 1 died 12 weeks after the beginning of the experiment from an undiagnosed cause. Another lamb from Group 2 died of urolithiasis 26 weeks after the commencement of the experiment.

The mean body weights of the lambs from Groups 1, 2 and 3 are shown in Fig. 3. Throughout the experimental period the body weights of the lambs of Groups 2 and 3 increased at a uniform rate, while those in Group 1 gained weight at a slightly lower rate. Two weeks after

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TABLE 3

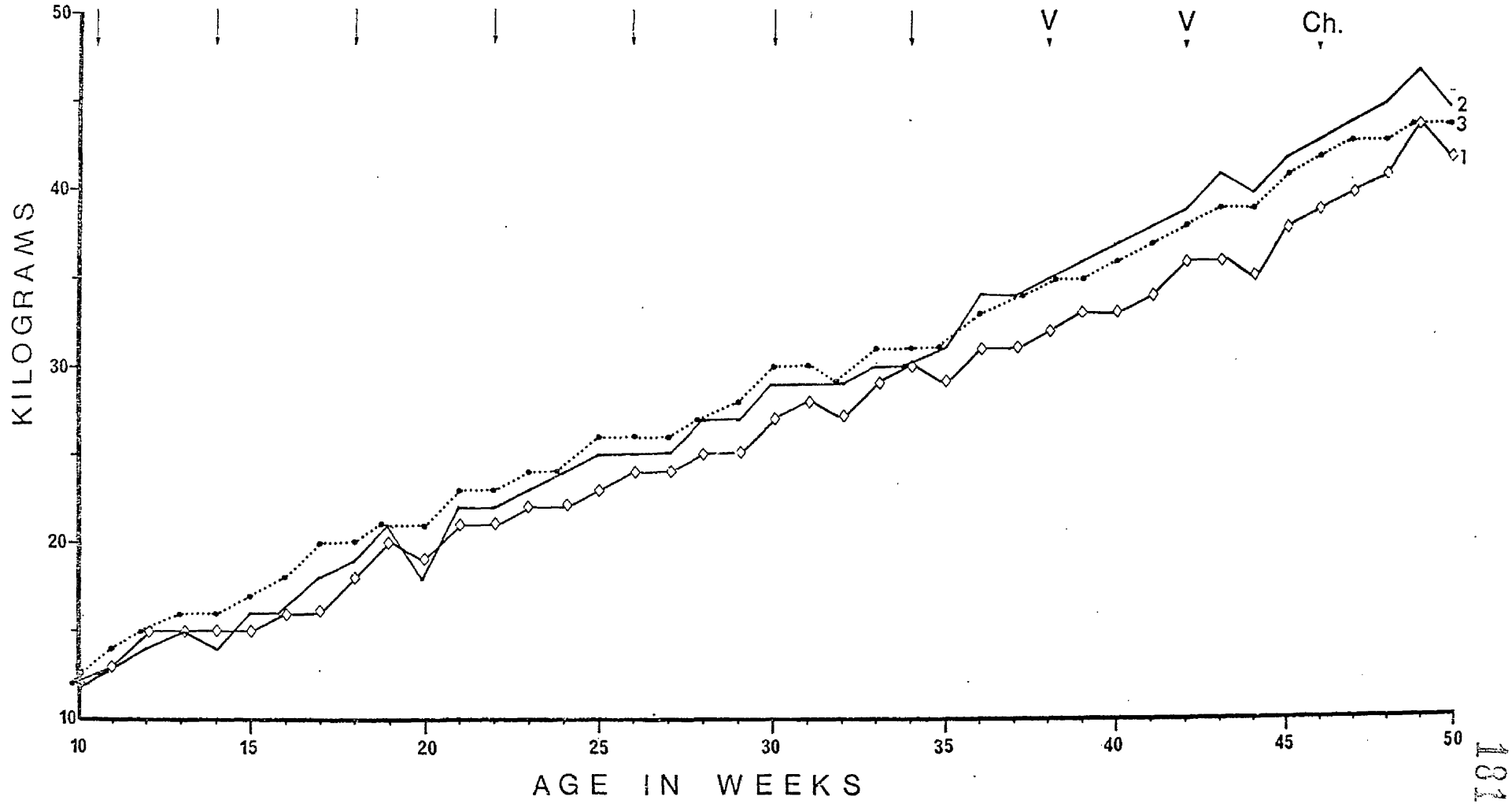
Design of Experiment 2 showing schedule of infection with normal (L_3) and γ-irradiated (γ-L_3) H. contortus larvae.

Group	No. of lambs	Age of Lambs 10	14	18	22	26	30	34	38	42	46	50
1	7	1,000 * L_3	1,000 * L_3	2,000 * L_3	2,000 * L_3	2,000 * L_3	2,000 * L_3	2,000 * L_3	10,000 * γ-L_3	10,000 * γ-L_3	10,000 L_3	K
2	7							*	10,000 * γ-L_3	10,000 * γ-L_3	10,000 L_3	K
3	7										10,000 L_3	K
4	1								10,000 γ-L_3	K		
5	1								10,000 L_3	K		
6	1									10,000 γ-L_3	K	
7	1									10,000 L_3	K	

* Anthelmintic treatment one week before each infection (thiabendazole).

K = Killed.

Figure 3 Mean body weights of 2 groups of lambs (Groups 1 and 2) immunised (V) with 2 x 10,000 γ-irradiated *H. contortus* L_3. Group 1 was previously exposed to infection with normal larvae (arrows) followed by anthelmintic 3 weeks later. These groups and the controls (Group 3) were challenged 4 weeks after second vaccination.



challenge the lambs of all 3 groups commenced losing weight. Individual body weights are recorded in Appendix C, Table 5.

Haematological Estimations

Packed cell volume percentages (PCV)

The mean PCV of lambs in Groups1 through 3 are shown in Fig. 4 and individual values in Appendix C, Table 6. In the lambs of Group 1 given infections of normal larvae at monthly intervals there was a minor drop in PCV after each infection followed by a recovery to former levels after anthelmintic therapy. Immunisation with the irradiated larvae caused a transient fall in PCV levels in the lambs in Groups 1 and 2, but this was again corrected by the anthelmintic treatment. Following challenge there was a progressive lowering of PCV until the time of slaughter when the mean values of the lambs in Groups 1, 2 and 3 were 28, 29 and 23% respectively.

Haemoglobin concentration and red blood cell counts

Since the fluctuation in haemoglobin levels and red blood cell counts were of a similar pattern to the PCV's these results have not been included.

Parasitological Data

Faecal egg counts (e.p.g.)

The individual number of H. contortus eggs per gram of faeces during the experimental period is shown in Appendix C, Table 7 and mean values in Fig. 5.

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Figure 4 Mean PCV of 2 groups of lambs (Groups 1 and 2) immunised (V) with 2 x 10,000 γ-irradiated *H. contortus* L_3. Group 1 was previously exposed to infection with normal larvae (arrows) followed by anthelmintic 3 weeks later. These groups and the controls (Group 3) were challenged 4 weeks after second vaccination.

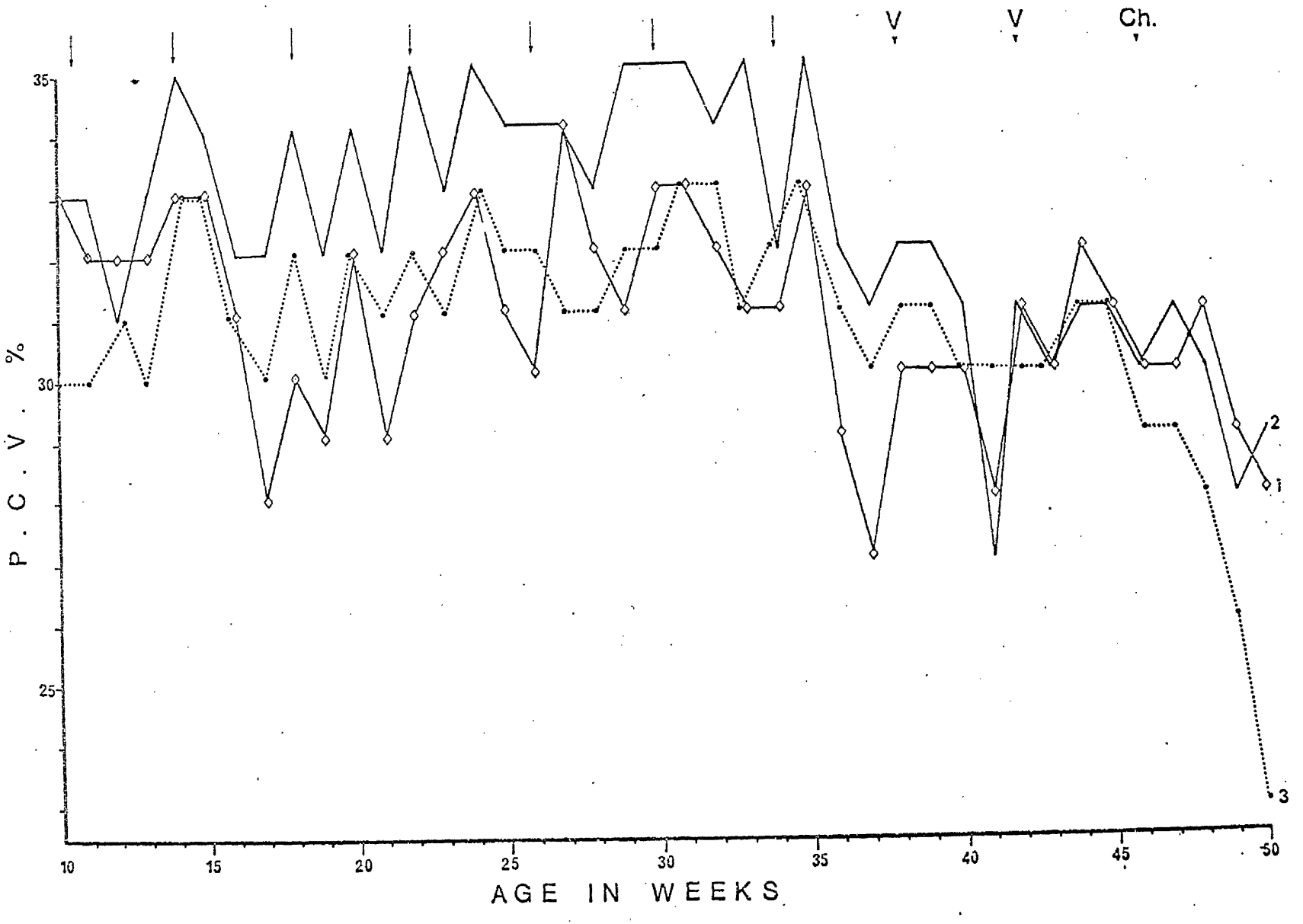
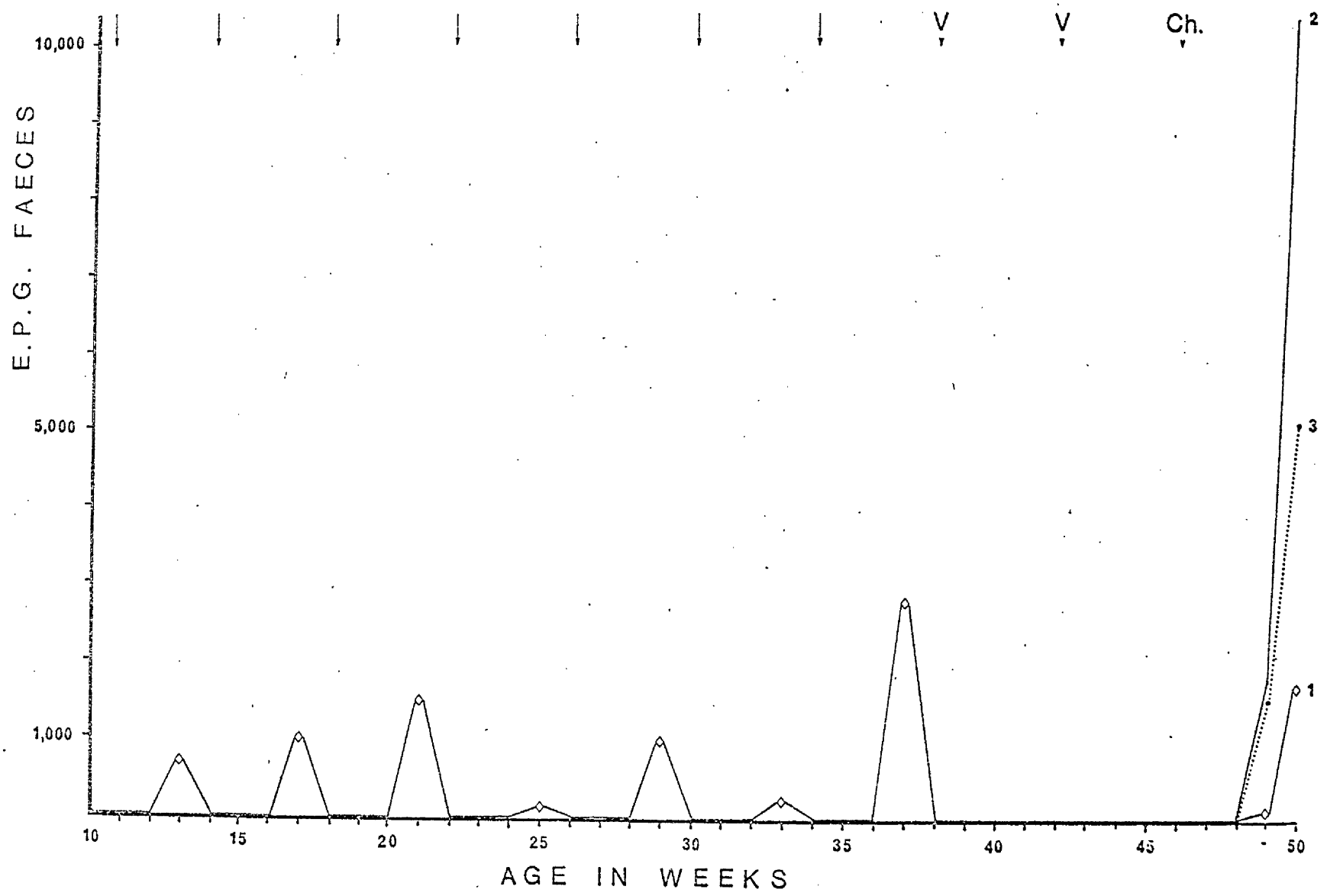


Figure 5 Mean faecal egg count of 2 groups of lambs (Groups 1 and 2) immunised (V) with 2 x 10,000 γ-irradiated H. contortus L_3. Group 1 was previously exposed to infection with normal larvae (arrows) followed by anthelmintic 3 weeks later. These groups and the controls (Group 3) were challenged 4 weeks after second vaccination.



In the lambs of Group 1, eggs were invariably present in the faeces by 3 weeks after each infection only to disappear again after each anthelmintic treatment. The numbers of eggs excreted by each lamb was extremely variable and ranged from 0 to 5,000. After the two immunising doses of irradiated larvae the faeces were consistently negative for H. contortus or other parasite ova. Following challenge only one lamb in Group 1 developed a positive faecal egg count. In Group 2, 4 lambs had counts ranging from 2,000-42,000 epg. during the 4th week post-challenge; the other lambs had 50 and 0 epg. respectively. In the control lambs, one remained negative for H. contortus eggs, while the remainder had faecal egg counts ranging from 1,000-13,000.

Worm recoveries

The mean numbers and standard error of H. contortus found in the lambs of the different groups are given in Table 4 and the individual worm counts in Appendix C, Table 8. All of the worms recovered were adults and arrested larvae were not present. The mean numbers of worms in the lambs of Groups 1, 2 and 3 were 717, 867 and 1,371 respectively. The differences between the numbers present in Group 3 and the lambs of the other groups are not significant.

In the lambs used to control the effectiveness of the irradiation procedure at the time of each vaccination 100 and 200 H. contortus were present. The lambs given normal larvae from the batch used to prepare the irradiated vaccine, had 2,900 and 1,900 worms following the

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TABLE 4

The mean number and standard error of H. contortus present at post-mortem of two groups of 9 month-old lambs immunised with irradiated larvae and subsequently challenged with normal larvae together with control lambs. One immunised group was previously exposed to infection with normal larvae from 10 weeks old.

Group	No. of Animals	Pre-immunisation treatment	Immunising Schedule with γ-irradiated larvae Week 38	Week 42	Challenge with Normal Larvae Week 46	Total	Male	Female	% Reduction compared with Controls
1	6 *	1-2,000 L_3 on week 10, 14, 18, 22, 26, 30 and 34	10,000	10,000	10,000 L_3	717±302	383±196	334±117	47.7
2	6 *	-	10,000	10,000	10,000 L_3	867±355	400±227	467±138	36.8
3	7	-	-	-	10,000 L_3	1371±454	671±200	700±257	
4	1	-	10,000	K		100	0	100	
5	1	-	10,000 Normal L_3	K		2900	1400	1500	
6	1	-	-	10,000	K	200	100	100	
7	1	-	-	10,000 Normal L_3	K	1900	1000	900	

* One lamb died before challenge.
Thiabendazole given one week prior to each larval administration in Groups 1, 2 and 3.

administration of 10,000 larvae.

The mean worm length of the H. contortus recovered from the lambs in Group 1 (monthly infections + anthelmintic, immunisation and challenge) was 16.64 ± 0.2 mm. The mean worm length collected from the lambs of Group 2 (immunised and challenged) and Group 3 lambs (only challenged) were 21.80 ± 0.6 and 20.36 ± 0.4 mm respectively. The differences in size between the worms of Group 1 lambs and those of Groups 2 and 3 was highly significant ($P > 0.001$).

EXPERIMENT 3

Experimental Design

Since the results of the second experiment were inexplicable in that vaccination of 9 month-old parasite-naive Scottish Blackface with H. contortus irradiated larvae failed to induce a satisfactory degree of immunity, in contrast to all previous reports[56,57,58,59], it was decided to repeat the experiment with minor alterations. To assess the possibility that anthelmintic therapy interfered with the host immune response an extra group of lambs was included which received 4-weekly infections of normal H. contortus larvae prior to immunisation but received only one anthelmintic treatment prior to vaccination. Furthermore, no anthelmintic was given once the immunisation programme had commenced. In the allocation of lambs to the various experimental groups the haemoglobin type (Hb type) was considered in addition to body weights. Although it would have been preferable, in view of the published results, to compare animals of HbA with the HbB type, few of the latter type were available and so lambs of HbAB type were used instead of HbB.

Apart from the above alterations the experimental design was the same as in the second experiment, and is presented in Table 5.

Observations

As in the previous two experiments all animals were observed daily. A more detailed examination was made at

TABLE 5

Design of Experiment 3 showing schedule of infection with normal (L_3) and γ-irradiated (γ-L_3) H. contortus larvae.

Group	No. of lambs	Age of Lambs in Weeks 10	14	18	22	26	30	34	38	42	46
1	7	1,000 * L_3	1,000 * L_3	2,000 * L_3	2,000 * L_3	2,000 * L_3	2,000 * L_3	10,000 γ-L_3	10,000 γ-L_3	10,000 L_3	K
2	8	1,000 L_3	1,000 L_3	2,000 L_3	2,000 L_3	2,000 L_3	2,000 * L_3	10,000 γ-L_3	10,000 γ-L_3	10,000 L_3	K
3	6						*	10,000 γ-L_3	10,000 γ-L_3	10,000 L_3	K
4	6						*			10,000 L_3	K
5	1							10,000 γ-L_3	K		
6	1							10,000 L_3	K		
7	1								10,000 γ-L_3	K	
8	1								10,000 L_3	K	

* Anthelmintic treatment one week before each infection (thiabendazole).

K = Killed.

weekly intervals, when the lambs were also weighed, blood was obtained for haematological studies (PCV, Hb and RBC) and rectal faecal samples collected for determinations of the numbers of H. contortus eggs per gram.

At post-mortem the abomasum was removed and the worm burden of the contents and mucosal digest determined. As in Experiment 2, the length of the female worms was measured using a planometer. A minimum of 20 females were measured from lambs of different groups and different Hb type; on two occasions this minimum number could not be obtained.

Results

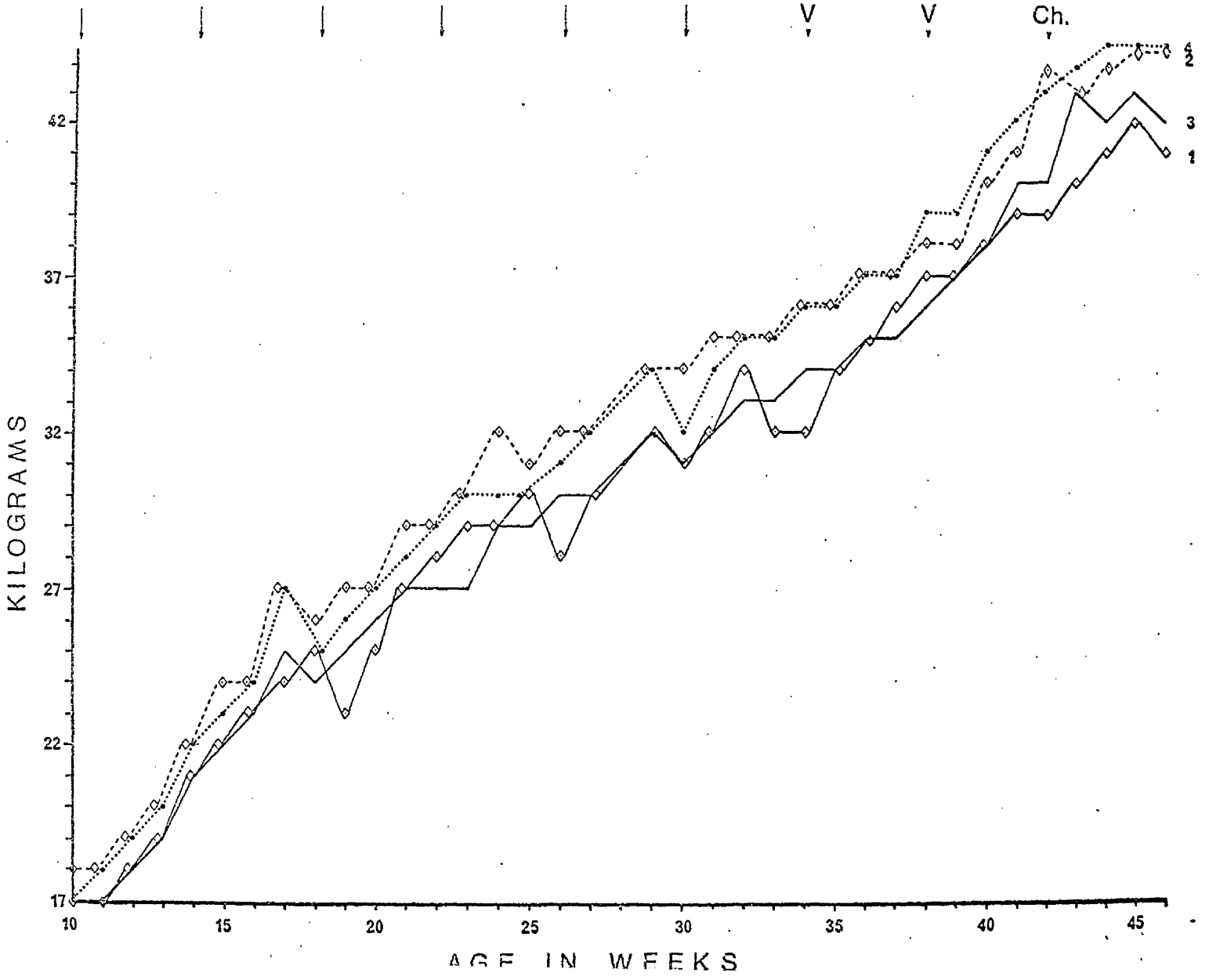
Clinical Data

One sheep of Group 1 (HbAB) and 2 of Group 2 (1 HbA and 1 HbAB) were killed prior to the pre-vaccination treatment of the animals of Groups 1, 2 and 3 to ascertain the worm burden at that point.

Throughout the experimental period the lambs gained weight steadily until the 4th week after challenge when a check occurred in all 4 groups; the differences in mean weight gains of the 4 groups were minor and not statistically significant.

Mean values are shown in Fig. 6 and individual weights are presented in Appendix C, Table 9.

Figure 6 Mean body weights of 5 groups of [illegible]
γ-irradiated H. contortus L_3. Groups 1 and 2 were previously exposed to normal infection followed by anthelmintic treatment in the case of Group 1. These groups plus the controls (Group 4) were challenged with 10,000 normal larvae 4 weeks after vaccination.



Haematological Estimations

Packed cell volume percentages (PCV)

The mean PCV of lambs of Groups 1, 2, 3 and 4 are shown in Fig. 7 and individual values are presented in Appendix C, Table 10. In lambs of Group 1, infected every 4 weeks with normal larvae, the PCV decreased gradually until 3 weeks later, then the values increased following the anthelmintic therapy. The first immunisation with irradiated larvae caused a drop to a mean of 26% and thereafter the percentages ranged from 26 to 28%. Animals of Group 2, given 1,000-2,000 normal L_3 every 4 weeks and no regular anthelmintic therapy had the lowest mean values (26-30%) until the single anthelmintic treatment prior to first vaccination, and thereafter had normal percentages. Following challenge these lambs showed a slight fall in PCV. Lambs of Group 3 had normal PCV (range 32-36%) until given the first dose of irradiated H. contortus L_3 and 4 weeks after the mean fell to 28%. No marked change was observed after the second immunisation and challenge. The PCV of the control lambs of Group 4 were between the normal ranges (30-35%) up to week 42 when they were challenged; thereafter the mean values declined to 24%. At the end of this experiment the mean PCV's were 26, 28, 30 and 24% in Groups 1, 2, 3 and 4 respectively.

Haemoglobin concentration and red blood cell counts

The haemoglobin concentration and red blood cell counts showed the same trend as the PCV and therefore these determinations will not be discussed further.

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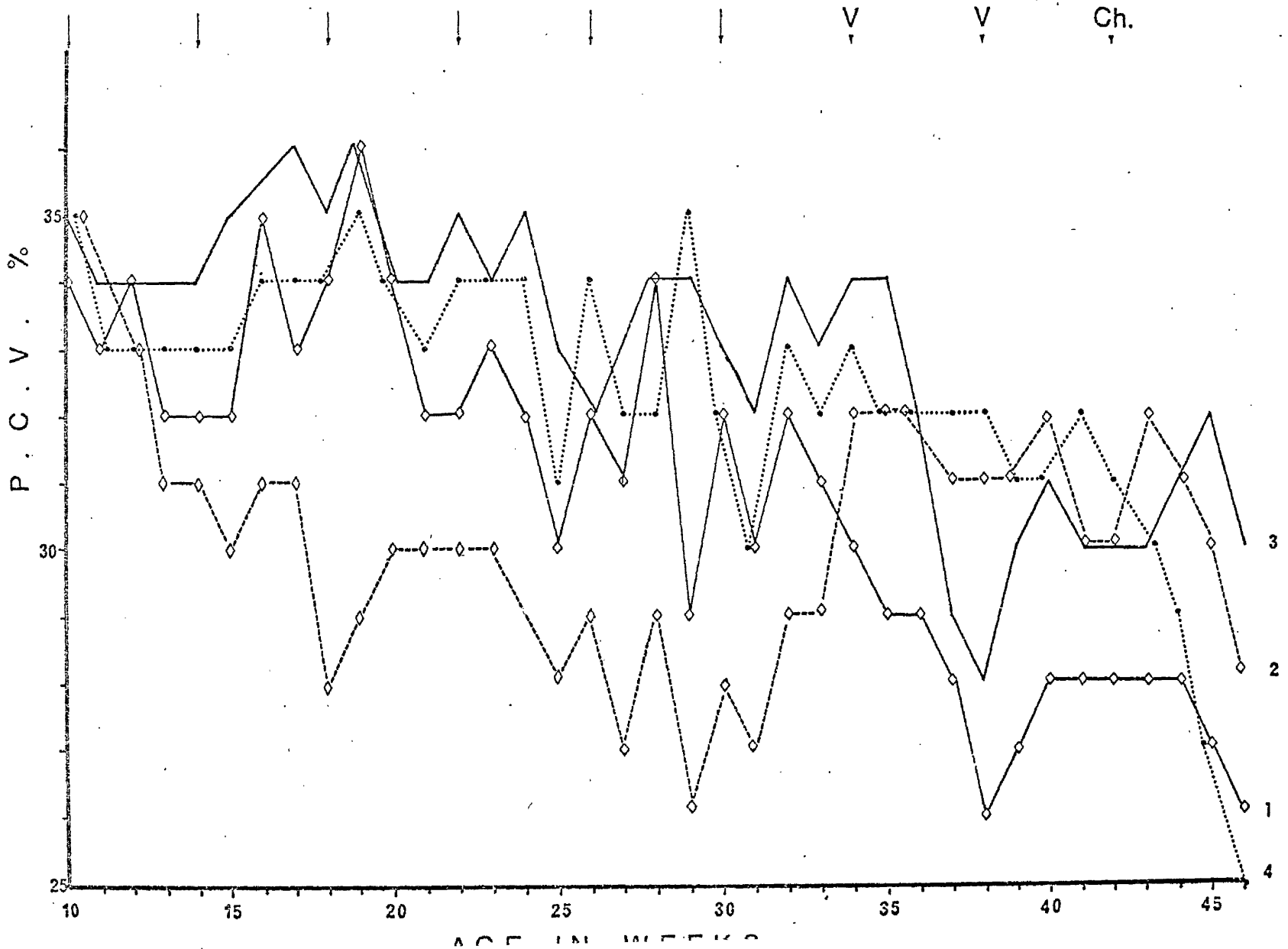
Figure 1. Mean PCV of 3 groups of lambs immunised (V) with 2 x 10,000 γ-irradiated *H. contortus* L_3. Groups 1 and 2 were previously exposed to normal infection followed by anthelmintic treatment in the case of Group 1. These groups plus the controls (Group 4) were challenged with 10,000 normal larvae 4 weeks after vaccination.



Faecal egg counts (e.p.g.)

The faecal egg counts of all animals were extremely variable. In Group 1, which received the spaced doses of normal larvae plus anthelmintic treatment, the mean weekly counts, when positive, ranged between 93 and 1,643 eggs per gram (epg.) during the pre-vaccination period. Following vaccination, only one animal was positive for H. contortus eggs on one occasion. By the 4th week after challenge 4 out of 6 lambs had eggs in their faeces, the mean epg. being 1,008. Sheep of Group 2 excreted eggs continuously starting from the 3rd week after the first spaced infection with normal larvae and the mean epg. ranged from 1,850 to 7,175. The excretion of eggs was halted by the anthelmintic treatment one week before the first vaccination. During the vaccination period all the faeces were consistently negative for nematode eggs. Four weeks after challenge the mean epg. was 417. In Group 3 the immunised lambs had a mean faecal egg count of 250 at 4 weeks after challenge, while the lambs of the control Group 4 had a mean of 5,525 epg. at 4 weeks after challenge. The mean epg. is shown in Fig. 8 and individual counts in Appendix C, Table 11.

Worm recoveries

The mean and standard error of the H. contortus burdens of the 4 main groups are shown in Table 6; the burdens of the irradiation and infectivity controls are also shown in this table. Individual H. contortus burdens are presented in Appendix C, Table 12.

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Figure 8 Mean epg. of 3 groups of lambs immunised (V) with 2 x 10,000 γ-irradiated *H. contortus* L_3. Groups 1 and 2 were previously exposed to normal infection followed by anthelmintic treatment in the case of Group 1. These groups plus the controls (Group 4) were challenged with 10,000 normal larvae 4 weeks after vaccination.

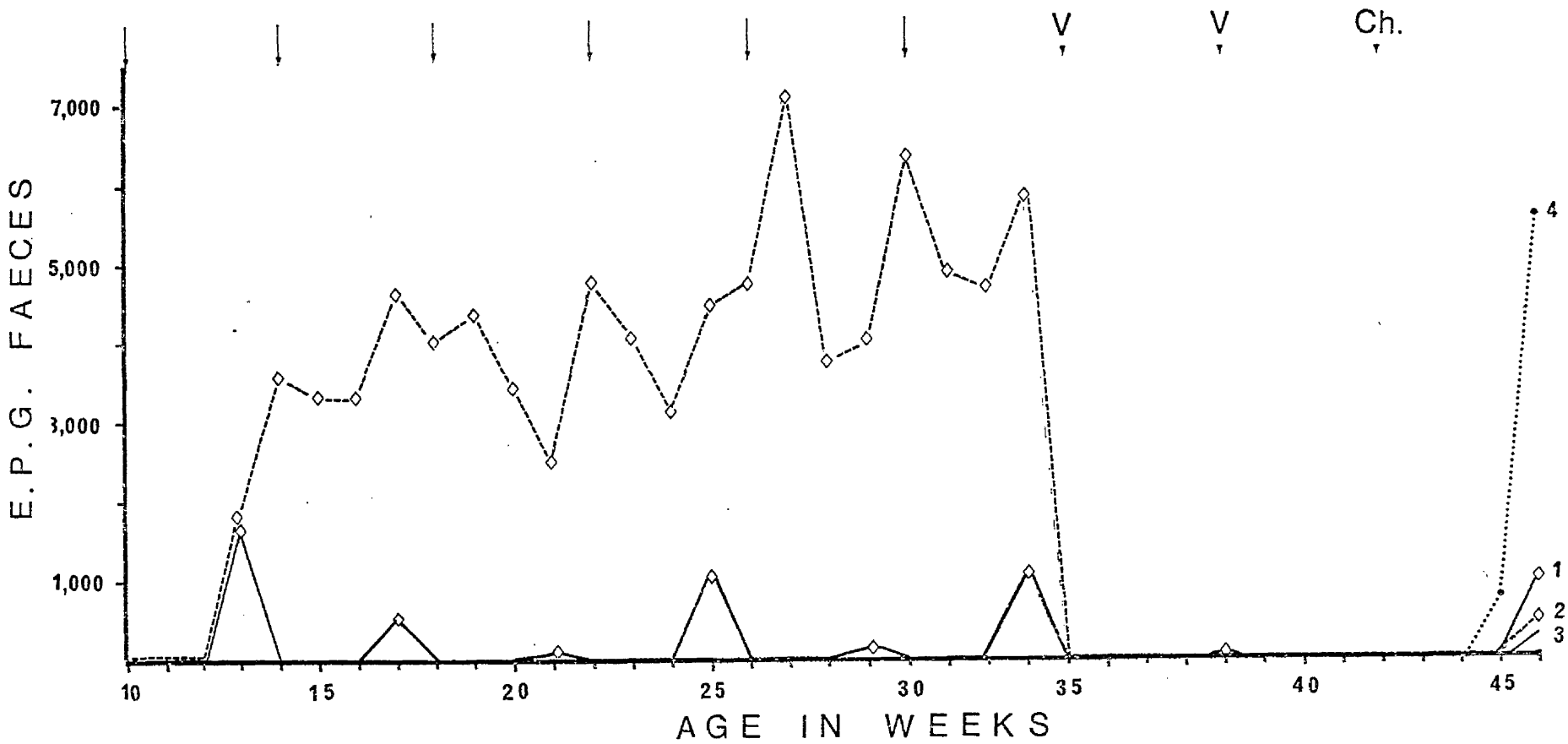

TABLE 6

The mean number and standard error of H. contortus present at post-mortem of three groups of 8½ month-old lambs immunised with irradiated larvae and subsequently challenged with normal larvae together with control lambs. Two immunised groups were previously exposed to infection with normal larvae from 10 weeks old, one receiving anthelmintic at 4-weekly intervals.

Group	No. of Animals	Pre-immunisation treatment	Immunising Schedule with γ-irradiated larvae			Challenge with Normal Larvae	Mean Number of Adult H. contortus on week 46			% Reduction compared with Controls
			Week 33	Week 34	Week 38	Week 42	Total	Male	Female	
1	6	1-2,000 L_3 on weeks 10, 14, 18, 22, 26 and 30. TBZ 3 weeks later.	TBZ	10,000	10,000	10,000 L_3	975±708	542±397	432±313	46.3
2	6	1-2,000 L_3 on weeks 10, 14, 18, 22, 26 and 30	TBZ	10,000	10,000	10,000 L_3	233±123	92± 64	142± 66	88.2
3	6	-	TBZ	10,000	10,000	10,000 L_3	100± 47	42± 27	58± 33	95.5
4	6	-	TBZ	-	-	10,000 L_3	1817±696	925±367	892±330	
5	1	-		10,000	K		150	0	150	
6	1	-		10,000 Normal L_3	K		1800	750	1050	
7	1	-			10,000	K	200	50	150	
8	1	-		-	10,000 Normal L_3	K	1650	750	900	

TBZ = thiabendazole K = Kill

No arrested development at the L_4 stage occurred and therefore the total population consisted exclusively of adults.

One lamb of Group 1 was killed before vaccination and 50 male worms were found at post-mortem. Two lambs of Group 2 were slaughtered at the same time to ascertain the worm population at this stage, and they each had a total of 950 adult H. contortus.

The mean numbers of worms in the 4 groups which were slaughtered 4 weeks after challenge, were: Group 1 - 975 ± 708; Group 2 - 233 ± 123; Group 3 - 100 ± 47; Group 4 - 1,817 ± 696 respectively. The differences in the worm burdens between the immunised Groups 2 and 3 and the controls (Group 4) were statistically significant ($P > 0.05$); the difference between the burdens of the other immunised Group 1 and the controls was not significant. The mean worm burden of Group 3 lambs was not statistically different from those of the lambs in Groups 1 and 2.

Groups 5 and 7 which were used to control the effectiveness of the irradiation at the time of each vaccination had a total of 150 and 200 H. contortus respectively. Groups 6 and 8 inoculated with 10,000 normal larvae from which the two batches of vaccine were prepared had 1,800 and 1,650 respectively.

No significant differences occurred in the worm burdens of lambs of different Hb type (see Appendix C, Table 12).

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The mean length in mm.of the female H. contortus worms recovered following challenge of each group of lambs and according to Hb type is shown in Table 7. Within each of the 4 principal experimental groups the mean lengths of the worms from HbA type lambs were not significantly different from those of HbAB type, except for Group 1. In fact, the length of the worms in each of the groups appeared to depend on the length of exposure of the lambs to H. contortus, i.e. the longer the exposure, the shorter the worms. These differences were highly significant between Group 1 and Groups 2 and 4 ($P > 0.001$) and significant between Groups 1 and 3 ($P > 0.05$).

DISCUSSION

Before discussing the results of the experiments from an immunological view point there are three other aspects which should be considered.

First, in Experiment 1, the numbers of attenuated larvae which developed were sufficient to result in a clinical anaemia in the lambs receiving immunising infections of 100,000 or 1,000,000 irradiated larvae (see Fig. 2). Since previous work has shown that higher doses of irradiation than used in this experiment produced overattenuation of H. contortus larvae and a poor immunogenic effect[56] it is apparent that large doses of H. contortus larvae irradiated at 60 Kr or less, even if successful in their immunogenic effect, would be contraindicated in view of the accompanying anaemia.

TABLE 7

Length in mm. of female worms recovered following challenge of lambs immunised against H. contortus and controls.

Group	Pre-immunisation treatment	Immunising Schedule with γ-irradiated larvae Week 33	Week 34	Week 38	Challenge with Normal Larvae Week 42	Hb Type	Number of Worms Measured	Mean Worm Length	Standard Error
1	1-2,000 L_3 on weeks 10, 14, 18, 22, 26 and 30. TBZ 3 weeks later	TBZ	10,000 γ-L_3	10,000 γ-L_3	10,000 L_3	A	36	14.11	0.3
						AB	54	15.80	0.3
2	1-2,000 L_3 on weeks 10, 14, 18, 22, 26 and 30	TBZ	10,000 γ-L_3	10,000 γ-L_3	10,000 L_3	A	6	12.00	1.2
						AB	40	14.10	0.5
3	-	TBZ	10,000 γ-L_3	10,000 γ-L_3	10,000 L_3	A	20	15.35	0.7
						AB	31	16.81	0.9
4	-	TBZ	-	-	10,000 L_3	A	27	16.74	0.6
						AB	18	17.17	0.6

TBZ = thiabendazole.

Secondly, at post-mortem of lambs from all three experiments the worm burdens consisted entirely of adult worms and arrested larval stages were not present. Although it is conceivable that arrested larval stages present were removed by the anthelmintic treatment prior to immunisation, this is unlikely, since arrested nematode larvae are notoriously resistant to anthelmintics[62] and at least some would have been present at post-mortem. A more likely explanation is that the strain of H. contortus used in these experiments, unlike that used by Dineen and his colleagues[26], does not possess the propensity for arrested development or alternatively has lost the ability to recognise the appropriate signal to arrest. Work with another abomasal nematode of cattle, Ostertagia ostertagi[63] has shown that a recently isolated field strain was capable of arrested development whereas a laboratory maintained strain was less inhibition prone. The technique used to maintain strains of nematodes in this laboratory is to culture faeces of donor animals for 2 weeks after eggs first appear in the faeces; it is, therefore, possible that some selection of the most rapidly maturing strains had taken place.

Thirdly, although sufficient lambs were not available to allow anthelmintic control groups to be included in the three experiments, there is sufficient evidence available to indicate that the efficiency of thiabendazole was extremely high. Thus, in Experiment 1, the clinical

response following treatment was immediate and blood values rapidly increased (Fig. 2); in Experiments 2 and 3 the lambs from Group 1 which received normal larvae at 4-weekly intervals and anthelmintic 3 weeks after each infection, the faecal egg counts became positive about 3 weeks after each infection only to become negative again after treatment. In contrast, the lambs of Group 2 in Experiment 3 which received regular doses of normal larvae but no anthelmintic, the faecal egg counts remained positive (Fig. 8).

From an immunological approach, the results of each of the three experiments are interesting, variable and in some instances inexplicable. In Experiment 1, the poor immunity engendered in Scottish Blackface lambs by the two immunising doses of irradiated H. contortus larvae, irrespective of dose size, is in agreement with several previous attempts to immunise young lambs with normal[47] or irradiated larvae[59,60] in various breeds of sheep including the Scottish Blackface. In the current investigation the large numbers of attenuated larvae used and the consequent anaemia may have influenced the outcome of the immunisation in that the immune response of lambs with severe anaemia is likely to be impaired. On the other hand the haematological status of the lambs improved dramatically following each anthelmintic treatment (see Fig. 2) with thiabendazole and at the time of

challenge they were not anaemic. It is also difficult to implicate the two anthelmintic treatments as influencing the results, since Christie and his colleagues[53], the only workers to produce a significant immunity to H. contortus in young Scottish Blackface lambs, also used two anthelmintic treatments between each series of 6 and 8 daily infection with normal larvae. However, although Christie's results are statistically significant there is a considerable scatter in the range of worm burdens they obtained, 3 out of the 12 immunised lambs having worm burdens of over 10,000 following challenge with 51,000 normal L_3.

In view of the present results and taking into consideration all the published data the author considers that immunisation of young lambs against H. contortus using normal or attenuated larvae alone is unlikely to be successful, irrespective of the size of the immunising dose. The reason for this is unknown, but the theory put forward by Urquhart and his colleagues[64] that it is due to immunological immaturity appears the most acceptable.

The main feature of the results in Experiment 2 was the failure to successfully immunise 9½ month-old Scottish Blackface lambs with two doses of irradiated H. contortus larvae. This result occurred not only in lambs which had been regularly exposed to normal H. contortus larvae prior

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to immunisation (Group 1) but also in lambs with no previous exposure to the parasite before immunisation (Group 2). The latter result is in complete contrast to previous studies by Jarrett et al.,[56,57], Bitakaramire[58] and Urquhart and his colleagues[59], the only difference in the current experiment being the administration of the anthelmintic thiabendazole 3 weeks after each immunising dose. The most likely explanation for these results is that not only does exposure to the parasite in early life interfere with subsequent immunisation but also that anthelmintic removal of the residual infections of irradiated worms impairs the immune response of lambs.

The results of Experiment 3 suggest that both of these hypotheses may be correct. Thus, the mean H. contortus burden in lambs immunised at 9 months old with two doses of 10,000 γ-irradiated larvae (but not given anthelmintic treatment as in Experiment 2) and subsequently challenged with 10,000 normal larvae (Group 3) was only 100 ± 47. In contrast, the non-immunised lambs given only the challenge infection (Group 4) of 10,000 larvae had a mean of 1,817 ± 696 worms (Table 6); the percentage reduction in worm burden of the vaccinates was therefore 95.5%. This result agrees with previous results of other workers[56, 57,58,59] using irradiated larvae and strongly suggests that the unusual result obtained in Experiment 2 was related to the anthelmintic treatments given to the immunised lambs.

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Of paramount interest were the results obtained in lambs exposed to 6 monthly doses of normal larvae with (Group 1) or without regular anthelmintic treatments (Group 2), followed by immunisation and challenge. The mean worm burdens in these two groups were 975 ± 708 and 233 ± 123 respectively indicating a mean reduction of 46 and 88 per cent respectively compared with the controls. When these results are compared with the 95 per cent reduction obtained in the immunised lambs of Group 3, it would appear that the regular exposure of lambs to normal larvae had interfered with their subsequent response to immunisation with irradiated larvae; when the doses of normal larvae were interspaced with anthelmintic therapy the degree of immunological impairment was greater. Either way, the hypothesis put forward by Lopez and Urquhart[35] that sheep are immunologically unresponsive to H. contortus infection if regularly exposed during early life, appears valid. A similar situation has been shown to exist with Nippostrongylus brasiliensis in the rat[65]. In these experiments rats given repeated infection of larvae from the neo-natal stage until well into adult life failed to develop fully the ability to expel their adult worm burdens which persisted for many weeks. In contrast, adult rats exposed to a similar infection schedule expelled their adult worm burdens in 2-4 weeks.

There was no clearly significant relationship between the Hb type of the immunised lambs and the number or length of worms established after challenge

(Table 7 and Appendix C, Table 12). This confirms previous studies by Radhakrishnan and his colleagues[42] and contemporaneous work by Altaif and Dargie (personal communication) who also failed to demonstrate any consistently significant relationship between worm length and Hb type. Instead the length of the female worms was inversely proportional to the duration of exposure to H. contortus infection.

A further parameter, namely the size and shape of the vulvular flap of the female worm is thought by Michel[66] to be affected by the hosts immune response while Daskalov[67] has attributed these differences mainly to the age of the worm. This criterion has not yet been examined in our material.

In conclusion, the results of these experiments have again confirmed the inability of young lambs to develop an effective immunity to H. contortus following immunisation with irradiated larvae. The reason for this is unknown and apart from its practical importance it seems to merit attention as a phenomenon of intrinsic immunological interest, since lambs of this age are readily immunised against a range of bacterial and viral infections.

The results also provide confirmation of the hypothesis that if lambs are exposed to H. contortus infections in early life an acquired unresponsiveness is superimposed on the age unresponsiveness and this persists throughout adult life. It seems likely that

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this sequence of events is responsible for the uniform susceptibility to H. contortus infection of adult sheep reared in endemic areas of the world.

Finally, an unexpected outcome of this work was the fact that repeated anthelmintic treatment of infected lambs appeared to exacerbate the state of unresponsiveness in adult life. Although this conclusion will require confirmation in a larger number of sheep it is interesting that two conflicting reports have recently appeared in the literature regarding the effect of anthelmintic treatment on the acquisition of immunity to O. circumcincta in sheep. Boag and Thomas[68] have reported that grazing lambs treated regularly with an anthelmintic had higher worm burdens at the end of the grazing season than untreated controls. In contrast, Reid and Armour[69], using the worm burden acquired from an experimental challenge as a measure of immunity, found no significant differences between the burdens of lambs previously treated with an anthelmintic and those of untreated lambs.

SUMMARY

In this section the respective roles of age, size of immunising dose, previous exposure to infection, anthelmintic therapy and Hb type on the immune response of Scottish Blackface sheep to irradiated H. contortus larvae were studied.

The age of lambs at the time of first immunisation was important, whereas the size of the immunising infection exerted no apparent influence. Thus, young parasite-free lambs immunised at 10 weeks-old and again at 14 weeks-old failed to develop a significant resistance to a challenge infection irrespective of whether the immunising dose consisted of 10,000, 100,000 or 1,000,000 third stage larvae γ-irradiated at 60 Kr. In contrast, parasite-free lambs first immunised at 9 months old and again at 10 months with 10,000 H. contortus larvae γ-irradiated at 60 Kr developed a highly significant immunity to a subsequent challenge, provided anthelmintic treatments were not given after the immunising doses.

There was strong experimental evidence that the administration of spaced doses of normal larvae to lambs for 6 months prior to immunisation with irradiated H. contortus larvae seriously impaired the immunity produced by vaccination. The reduction in immunity was greater when anthelmintics were administered after each larval infection.

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Hb type did not significantly alter the immunity produced by irradiated larvae as measured by worm burden established after challenge.

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GENERAL SUMMARY

The study in this thesis were concerned with investigations into 3 separate problems which have arisen in the development of immunisation procedures against 3 separate and serious helminth diseases of cattle and sheep. The comparative efficiency of two techniques used to monitor the number of nematode larvae on herbage was also examined.

In Section 1, the previously unresolved problem concerning the immunisation of young milk-fed calves against the lungworm Dictyocaulus viviparus was investigated. Successful immunisation of weaned calves aged at least 8 weeks with two doses of X-ray attenuated infective larvae has been practised in Britain and Western Europe for some years now and the vaccine is commercially available as Dictol. The fact that immunisation with Dictol is not recommended until the weaned calves are 8 weeks-old has precluded the successful control of lungworm disease in dairy herds where calves are grazed from an early age and in beef herds where calves are suckled for several months.

In the current studies, when Dictol was administered to pail-fed milk calves at 3 and 7 weeks of age the resistance to a subsequent experimental challenge 4 weeks later was excellent compared with non-immunised controls. As judged by the criteria of clinical signs, serological response and post-mortem lungworm burdens the immunity acquired by these young calves was comparable to that obtained in calves immunised at 8 and 12 weeks-old.

When the immunisation procedure was repeated in suckled calves aged 3 and 7 weeks the degree of resistance to subsequent challenge was good but inferior to that obtained in pail-fed milk calves. Unfortunately, the situation was complicated by the presence of a Mycoplasma infection in the lungs of these calves, and it is possible that this and/or the blocking effect of maternal antibody may have influenced the result.

Nevertheless, in the absence of any concurrent lung infection these experiments indicate that Dictol immunisation of young calves on a milk diet, whether suckled or pail-fed, appears to be a practical proposition and may result in more widespread and effective control of lungworm disease.

In Section 2, immunisation with γ-irradiated larvae of young calves against the abomasal nematode Ostertagia ostertagi was re-investigated. Previous studies had shown that the acquisition of a solid immunity to this parasite was slow and at best it was hoped that immunisation would limit the infection acquired to a tolerable level. In 1973, parasite-naive calves aged 8 weeks-old were immunised with 2 doses of 100,000 O. ostertagi larvae γ-irradiated at 60 Kr and administered orally at an interval of 4 weeks. When subsequently grazed on pasture with a high level of O. ostertagi infection the calves failed to develop a significant resistance and clinical disease of an intensity similar

to the controls developed in the immunised calves. In 1974, the immunisation procedure was repeated when the initial level of pasture infection was low. Although some immunised calves developed mild ostertagiasis, a moderating effect of the vaccine was apparent in that these calves acquired lower worm burdens than the controls, the clinical disease was milder and the subsequent level of infection on the pasture was lower than in the area grazed by the controls. It is doubtful however if this form of immunisation has any practical value for the control of ostertagiasis.

An interesting feature of both experiments was the apparent suppression of Dictol-induced immunity in calves which had prior exposure to severe ostertagiasis.

In Section 3, the pasture levels of O. ostertagi and D. viviparus larvae were monitored and compared using two techniques, one involving sieving and filtration of the herbage washings, and the other repeated sedimentation of the washings; recovery of both species was approximately similar when over 100 L_3/Kg of herbage were present.

Finally, in Section 4 some factors affecting vaccination of lambs against the stomach worm Haemonchus contortus with larvae attenuated by γ-rays were studied. The results confirmed the inability of parasite-naive young lambs, aged 3 months to develop any immunity to challenge with H. contortus following the administration of 2 doses of attenuated larvae at an interval of 4 weeks. This poor response to immunisation occurred independent of the size of the immunising dose. When older parasite-naive lambs

aged 9 to 10 months were also vaccinated with 2 doses of 10,000 larvae previously subjected to γ-irradiation at 60 Kr they developed a highly significant resistance to an experimental challenge 4 weeks later. If however these older lambs were previously exposed to regular infection with normal larvae from an early age the subsequent response to immunisation was impaired. Repeated anthelmintic therapy during the period of larval exposure appeared to exaggerate the unresponsiveness to subsequent immunisation. These observations on the host/parasite relationship occurred independently of the haemoglobin type of the lambs involved.

The innate unresponsiveness of young lambs plus the superimposed acquired unresponsiveness of lambs exposed to infection with H. contortus in early life makes any immediate prospect of vaccination improbable in areas where the parasite is endemic.

APPENDICES.

APPENDIX A TABLE 1

Respiratory rates of individual milk-fed calves immunised with Dictol (Group 1) at 3 and 7 weeks of age and their Controls (Group 2). Both groups were challenged on week 11.

Days after Vaccination		Group 1						Group 2					
		192	194	195	201	203	Mean	190	191	200	206	236	Mean
1	V	20	28	30	21	23	24	19	24	27	28	23	24
2		24	24	27	26	30	26	24	21	23	30	27	25
3		24	23	28	24	28	25	24	25	27	25	26	25
4		27	29	23	25	24	26	25	23	28	24	26	25
5		28	27	26	24	25	26	29	22	26	21	23	24
6		24	29	31	20	24	26	27	26	23	24	30	26
7		28	33	37	29	28	31	26	28	24	27	23	26
8		32	37	49	32	32	36	26	29	25	24	24	26
11		36	39	48	36	47	41	27	26	28	24	25	26
12		48	45	51	36	56	47	27	25	33	21	24	26
13		49	47	48	38	50	46	28	28	25	23	23	26
14		38	45	47	40	52	44	25	31	20	24	28	26
15		48	50	52	52	48	50	28	28	24	26	28	27
16		50	48	56	48	50	50	28	28	30	28	30	29
17		58	55	64	60	56	59	32	26	36	28	40	32
18		50	50	58	40	58	51	29	30	28	24	30	28
19		52	48	56	36	56	50	32	36	28	30	36	32
20		48	50	50	38	50	47	28	30	30	24	30	28
21		52	48	52	36	52	48	24	32	32	24	32	29
22		56	40	50	60	56	52	26	30	30	26	36	30
23		50	44	56	56	54	52	29	29	32	30	28	29
24		60	39	50	64	50	53	26	30	30	30	28	29
25		52	36	48	76	60	54	33	32	28	32	36	32
26		68	48	44	50	60	54	28	36	32	28	36	32
27		48	52	52	72	44	54	28	36	24	28	32	30
28	V	40	52	36	76	40	49	24	28	20	24	28	25
29		44	48	40	68	48	50	26	32	22	32	32	29
30		40	50	46	60	46	48	28	30	26	28	26	28
31		46	48	42	64	44	49	30	28	28	27	28	28
32		44	46	44	52	40	45	26	28	24	26	30	27
33		48	44	40	60	50	48	24	32	28	32	32	30
34		44	46	44	56	50	48	28	32	30	30	28	30
35		40	44	40	64	54	48	30	28	28	30	27	29
36		40	48	42	84	54	54	32	33	28	29	30	30
37		48	50	46	80	50	55	30	32	30	28	26	29
39		60	48	52	84	44	58	30	36	32	32	32	32
40		52	38	36	48	36	42	32	32	24	20	24	26
41		60	32	38	72	32	47	24	32	20	24	32	26
42		48	32	32	52	28	38	28	32	28	20	22	26
43		52	36	36	50	36	42	26	30	26	24	20	25
44		50	38	30	48	30	39	24	32	24	26	24	26
45		48	30	36	50	28	38	26	28	28	24	26	26

APPENDIX A, TABLE 1 (Cont'd.)

Days after Vaccination		Group 1						Group 2					
		192	194	195	201	203	Mean	190	191	200	206	236	Mean
46		66	32	28	52	24	40	20	32	24	20	24	24
47		48	40	24	52	28	38	20	32	24	20	30	25
48		50	40	28	48	32	40	20	24	20	20	24	22
49		52	28	24	40	28	34	20	24	22	24	26	23
50		56	40	30	54	32	42	24	26	24	28	28	26
51		52	42	30	50	30	41	26	24	26	26	26	26
52		48	36	28	44	30	37	24	28	28	28	26	27
53		40	32	28	36	32	34	24	26	24	26	28	26
54		40	36	32	36	30	35	26	28	26	28	20	26
55		44	30	32	30	36	34	28	24	20	20	20	22
56	Ch.	42	28	28	32	28	32	26	24	26	28	24	26
57		26	28	26	32	26	28	24	24	20	-	20	22
58		30	32	28	30	28	30	26	24	24	-	24	24
59		24	30	28	36	30	30	24	28	24	-	24	25
60		24	32	24	64	40	37	24	32	20	-	24	25
61		32	36	28	40	36	34	24	26	24	-	24	24
62		32	32	20	48	36	34	24	28	20	-	24	24
63		30	28	32	48	36	35	30	28	36	-	24	29
64		32	32	40	56	48	42	36	36	40	-	28	35
65		36	32	44	56	48	43	40	38	40	-	32	37
66		40	40	40	60	56	47	44	36	36	-	36	38
67		38	36	32	48	40	39	32	36	48	-	32	37
68		30	44	24	52	60	42	36	48	46	-	36	41
69		36	40	28	52	52	42	40	52	56	-	48	49
70		48	36	28	56	48	43	48	60	56	-	52	54
71		60	28	28	60	40	43	48	60	68	-	48	56
72		36	32	32	64	48	42	40	52	68	-	88	62
73		40	36	30	60	40	41	40	56	76	-	80	63
74		38	36	36	72	44	45	42	48	70	-	70	57
75		36	38	36	76	48	47	40	56	72	-	76	61
76		44	44	36	84	56	53	44	68	80	-	64	64
77		48	48	32	92	64	57	52	76	80	-	60	67
78		48	60	36	72	72	58	60	76	84	-	80	75
79		40	56	36	76	68	55	56	84	84	-	88	78
80		44	44	32	92	64	55	60	88	76	-	88	78
81		40	44	36	80	72	54	64	84	88	-	76	78
82		48	34	32	68	68	50	60	84	80	-	88	78
83		40	36	30	70	64	48	60	92	88	-	92	83
84		40	38	32	60	64	47	72	84	84	-	100	85
85		40	40	28	68	68	49	68	80	76	-	112	84
86		40	30	28	68	76	48	84	84	80	-	116	91

APPENDIX A TABLE 2

Respiratory rates of individual milk-fed calves immunised with Dictol (Group 3) at 8 and 12 weeks of age and their controls (Group 4). Both groups were challenged on week 16.

Days after Vaccination		Group 3						Group 4					
		193	197	199	202	237	Mean	205	232	233	234	235	Mean
0	V	24	30	32	32	32	30	28	32	24	36	30	30
1		32	32	32	36	32	33	40	32	36	36	28	34
2		30	30	32	30	28	30	32	30	36	30	32	32
3		28	26	30	32	34	30	30	30	34	30	32	31
4		26	32	36	36	30	32	30	32	36	28	30	31
5		28	29	36	32	30	31	28	24	32	28	30	28
7		28	36	40	36	36	35	28	28	30	33	30	30
8		28	28	44	32	36	34	32	28	36	32	24	30
9		26	36	28	28	26	29	24	24	40	24	22	27
10		32	48	36	40	32	38	28	28	40	26	20	28
11		36	44	38	48	36	40	24	26	36	24	20	26
12		38	40	42	36	40	39	26	24	40	28	24	28
13		36	40	40	38	36	38	28	24	38	26	26	28
14		40	28	60	32	28	38	24	20	44	32	28	30
15		32	24	52	46	36	38	20	20	44	24	18	25
16		32	28	46	44	38	38	22	20	30	20	18	22
17		28	32	40	32	28	32	20	22	36	24	20	24
18		28	56	46	60	40	46	20	24	40	24	20	26
19		32	40	46	52	36	41	24	28	40	26	26	29
20		36	36	44	50	44	42	20	26	36	24	24	26
21		48	32	40	32	40	38	24	28	36	26	24	28
22		46	36	40	30	40	38	20	26	36	24	20	25
23		40	40	38	32	36	37	20	28	32	20	26	25
24		24	40	36	28	32	32	24	26	32	24	26	26
25		36	36	38	32	36	36	26	24	30	26	24	26
26		30	36	36	36	30	34	24	24	28	24	20	24
27	V	32	36	36	48	32	37	28	26	28	24	24	26
28		30	30	36	40	30	33	20	28	28	20	20	23
29		32	28	38	44	36	36	24	20	30	24	28	25
30		30	26	40	36	30	32	28	28	26	24	20	25
31		28	24	48	36	48	37	20	36	30	20	24	26
32		28	28	26	30	44	31	20	24	28	26	24	24
33		28	26	28	32	40	31	24	20	30	28	26	26
34		28	36	32	32	42	34	28	24	30	30	28	28
35		44	40	44	28	40	39	28	28	32	30	28	29
36		44	44	40	36	40	41	26	28	28	30	26	28
37		48	44	40	40	48	44	26	32	28	28	28	28
38		46	40	36	44	44	42	24	32	28	26	28	28
39		32	32	60	36	32	38	28	26	24	28	24	26
40		30	32	48	40	30	36	24	28	26	24	26	26
41		28	32	40	42	34	35	26	28	28	24	28	27

APPENDIX A, TABLE 2 (Cont'd.)

Days after Vaccination		Group 3						Group 4					
		193	197	199	202	237	Mean	205	232	233	234	235	Mean
42		28	32	40	40	32	34	24	26	28	28	26	26
43		28	24	40	32	36	32	24	22	34	28	32	28
44		20	28	36	36	30	30	24	24	32	26	30	27
45		24	26	36	32	32	30	24	28	28	28	32	28
46		28	28	40	30	36	32	20	28	30	24	32	27
47		24	20	48	36	36	33	24	26	28	28	30	27
48		36	28	48	38	36	37	26	28	28	24	28	27
49		24	24	40	32	32	30	30	32	28	32	24	29
50		28	26	40	30	32	31	30	30	28	30	24	28
51		26	24	48	30	32	32	30	32	32	32	28	31
52		36	36	44	48	44	42	28	28	36	24	28	29
53		32	36	34	36	38	35	28	24	26	20	20	24
54		36	36	32	32	32	34	24	28	28	24	20	25
55		36	36	28	38	30	34	24	28	26	28	28	27
56	Ch.	40	36	36	38	36	37	24	34	28	28	24	28
58		28	38	32	36	44	36	32	44	48	32	30	37
59		24	48	32	38	40	36	28	26	36	36	36	32
60		28	40	36	36	38	36	24	26	36	40	40	33
61		28	44	36	36	36	36	28	28	36	32	40	33
62		32	38	44	30	34	36	24	26	32	24	40	29
63		32	36	40	32	36	35	24	32	36	28	36	31
64		36	36	56	40	44	42	28	44	36	36	24	34
65		36	64	36	36	48	44	40	48	44	26	28	37
66		36	36	52	36	52	42	28	38	52	36	36	38
67		44	36	44	48	48	44	48	40	68	56	48	52
68		40	36	40	44	44	41	62	56	56	64	68	61
69		36	32	44	44	48	41	84	76	48	72	74	71
70		48	40	40	48	60	47	80	88	80	80	68	79
71		32	40	38	60	72	48	72	88	108	80	-	87
72		44	28	36	52	56	43	68	92	96	84	-	85
73		40	36	36	48	52	42	72	100	104	80	-	89
74		40	40	36	48	50	43	68	96	100	80	-	86
75		40	48	40	52	50	46	68	92	104	80	-	86
76		32	34	52	64	56	48	72	88	100	100	-	90
77		28	32	56	64	52	46	84	96	108	80	-	92
78		24	26	48	68	64	46	76	88	108	80	-	88
79		28	28	48	68	60	46	72	96	104	88	-	90
80		28	32	40	64	68	46	76	100	108	92	-	94
81		28	28	40	68	72	47	80	104	104	96	-	96
82		36	28	44	60	72	48	72	100	112	96	-	95
83		32	28	40	56	68	45	80	96	108	100	-	96
84		28	28	40	48	80	45	88	100	112	96	-	99
85		28	28	48	56	68	46	80	112	108	80	-	95
86		26	24	40	52	68	42	88	108	112	84	-	98

APPENDIX A TABLE 3

Body weights in kilograms of individual milk-fed calves immunised with Dictol (Groups 1 and 3) and their controls (Groups 2 and 4). The calves of Group 1 were vaccinated at 3 and 7 weeks-old and those of Group 3 at 8 and 12 weeks-old. Challenge was at 4 weeks after vaccination.

Age	Group 1.					Mean	Group 2.					Mean
W	192	194	195	201	203	S.E.	190	191	200	206	236	S.E.
0	33	30	31	36	33	33±1.0	28	28	30	39	32	31±2.0
1	34	33	34	37	33	34±0.7	29	27	30	40	36	32±2.4
2	37	34	38	35	39	37±0.9	33	29	32	40	37	34±1.9
3	43	37	43	37	44	41±1.6	36	35	37	47	40	39±2.2
4	48	40	46	37	48	44±2.2	39	37	40	46	45	41±1.7
5	55	41	53	38	52	48±3.5	44	42	45	48	50	46±1.4
6	59	44	59	39	56	51±4.1	46	47	48	51	55	49±1.6
7	64	46	65	43	63	56±4.8	52	53	52	57	65	56±2.5
8	66	50	71	45	69	60±5.3	57	57	55	57	70	59±2.7
9	71	54	72	48	66	62±4.8	57	56	57	61	72	61±3.0
10	70	55	75	49	73	64±5.2	59	57	59	62	74	62±3.1
11	73	57	77	52	76	67±5.2	64	60	62	64	77	65±3.0
12	80	60	79	54	78	70±5.5	69	60	63	-	81	68±4.6
13	84	64	81	56	80	73±5.5	70	63	65	-	84	70±4.7
14	85	65	83	56	82	74±5.8	69	62	65	-	83	70±4.6
15	87	65	82	56	87	75±6.3	67	61	64	-	80	68±4.2

Age	Group 3.					Mean	Group 4.					Mean
W	193	197	199	202	237	S.E.	205	232	233	234	235	S.E.
0	31	40	37	35	32	35±1.6	33	36	43	34	29	35±2.3
1	35	40	37	36	33	36±1.2	38	34	46	35	31	37±2.6
2	38	45	43	38	35	40±1.8	42	35	48	38	34	39±2.6
3	43	50	48	42	39	44±2.0	48	38	55	43	37	44±3.3
4	45	55	53	45	40	48±2.8	55	41	60	46	39	48±4.0
5	50	63	60	49	44	53±3.6	63	50	66	50	42	54±4.5
6	53	67	65	55	50	58±3.4	70	50	71	56	50	59±4.7
7	61	77	70	60	53	64	80	55	80	63	50	66±6.2
8	68	85	76	65	59	71±4.5	85	56	84	68	51	69±7.0
9	67	89	79	66	60	72±5.2	91	60	86	72	48	71±8.0
10	69	88	81	70	62	74±4.6	93	62	90	72	50	73±8.2
11	75	90	86	74	65	78±4.5	96	63	92	75	54	76±8.1
12	78	94	89	75	66	80±5.0	98	66	93	78	56	78±7.9
13	81	96	91	75	65	82±5.5	100	70	95	79	59	81±7.6
14	85	100	96	80	69	86±5.6	105	72	100	82	62	84±8.1
15	87	102	97	84	73	89±5.1	106	76	105	85	64	87±8.2
16	91	106	100	87	78	92±4.9	108	79	111	88	67	91±8.4
17	95	110	101	91	80	95±5.0	109	80	109	86	64	90±8.7
18	99	114	102	92	79	97±5.8	108	80	105	87	-	95±6.8
19	102	116	102	94	75	98±6.7	106	77	103	83	-	92±7.2
20	101	119	105	95	81	100±6.2	105	75	102	82	-	91±7.4

APPENDIX A TABLE 4

Larvae per gram of faeces of individual milk-fed calves immunised with Dictol (Groups 1 and 3) and their controls (Groups 2 and 4). The calves of Group 1 were vaccinated at 3 and 7 weeks-old and those of Group 3 at 8 and 12 weeks-old. Challenge was at 4 weeks after vaccination.

Age	Group 1.					Group 2.				
Weeks	192	194	195	201	203	190	191	200	236	Mean
0	-	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	-	-	-	
2	-	-	-	-	-	-	-	-	-	
3	-	-	-	-	-	-	-	-	-	
4	-	-	-	-	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-	
6	-	-	-	-	-	-	-	-	-	
7	-	-	-	-	-	-	-	-	-	
8	-	-	-	-	-	-	-	-	-	
9	-	-	-	-	-	-	-	-	-	
10	-	-	-	-	-	-	-	-	-	
11	-	-	-	-	-	-	-	-	-	
12	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-	
15	-	-	-	-	-	1,200	400	1,600	850	1,012

Age	Group 3					Group 4.					
Weeks	193	197	199	202	237	205	232	233	234	235	Mean
0	-	-	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	-	-	-	-	
2	-	-	-	-	-	-	-	-	-	-	
3	-	-	-	-	-	-	-	-	-	-	
4	-	-	-	-	-	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-	-	
6	-	-	-	-	-	-	-	-	-	-	
7	-	-	-	-	-	-	-	-	-	-	
8	-	-	-	-	-	-	-	-	-	-	
9	-	-	-	-	-	-	-	-	-	-	
10	-	-	-	-	-	-	-	-	-	-	
11	-	-	-	-	-	-	-	-	-	-	
12	-	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-	-	
15	-	-	-	-	-	-	-	-	-	-	
16	-	-	-	-	-	-	-	-	-	-	
17	-	-	-	-	-	-	-	-	-	-	
18	-	-	-	-	-	-	-	-	-	-	
19	-	-	-	-	-	-	-	-	-	-	
20	-	-	-	-	-	1,100	200	200	100		400

APPENDIX A TABLE 5

Haemagglutination titres of individual milk-fed calves immunised with Dictol (Groups 1 and 3) and their controls (Groups 2 and 4). The calves of Group 1 were vaccinated at 3 and 7 weeks-old and those of Group 3 at 8 and 12 weeks-old. Challenge was at 4 weeks after vaccination.

Weeks after Vaccination	Group 1. 192	194	195	201	203	Mean S.E.	Group 2. 190	191	200	236	Mean S.E.
0	1	2	1	3	1	1.6±0.4	-	-	-	-	-
1	2	2	2	1	1	1.6±0.2	-	-	-	-	-
2	2	1	1	2	1	1.4±0.2	-	-	-	-	-
3	1	1	1	1	3	1.4±0.4	-	-	-	-	-
4	2	2	3	4	1	2.4±0.5	-	-	-	-	-
5	5	7	7	6	3	5.6±0.7	-	-	-	-	-
6	6	6	11	9	6	7.6±1.0	-	-	-	-	-
7	4	4	8	3	3	4.4±0.9	-	-	-	-	-
8	3	3	4	5	2	3.4±0.5	1	2	1	1	1.2±0.2
9	3	2	7	6	4	4.5±0.9	1	1	1	1	1.0
10	6	5	6	8	7	6.4±0.5	1	1	2	3	1.7±0.5
11	6	6	7	10	8	7.4±0.7	4	5	5	3	4.2±0.5
12	7	8	9	9	8	8.2±0.4	6	8	7	5	6.5±0.6

Weeks after Vaccination	Group 3. 193	197	199	203	237	Mean S.E.	Group 4. 205	232	233	234	235	Mean S.E.
0	3	0	3	3	3	2.4±0.6	-	-	-	-	-	-
1	4	1	2	1	2	2.0±0.5	-	-	-	-	-	-
2	3	1	1	1	2	1.4±0.4	-	-	-	-	-	-
3	2	2	3	2	3	2.4±0.2	-	-	-	-	-	-
4	5	4	4	1	3	3.4±0.7	-	-	-	-	-	-
5	3	4	2	2	3	2.8±0.4	-	-	-	-	-	-
6	5	7	3	3	5	4.6±0.7	-	-	-	-	-	-
7	6	10	6	6	3	6.2±1.1	-	-	-	-	-	-
8	7	10	7	7	6	7.4±0.7	2	2	4	3	4	0.4
9	8	10	8	7	7	8.0±0.5	3	1	0	3	2	1.8±0.6
10	10	10	9	8	6	8.6±0.7	5	4	3	2	Died	3.5±0.6
11	10	10	10	9	7	9.2±0.6	6	9	8	5	-	7.0±0.9
12	11	10	9	10	4	8.8±1.2	8	9	7	5	-	7.2±0.9

APPENDIX A TABLE 6

Respiratory rates of individual suckled calves immunised with Dictol (Group 1) at 3 and 7 weeks of age and their controls (Group 2). Both groups were challenged on week 11.

Days after Vaccination		Group 1.							Group 2.						
		1	3	5	6	9	12	Mean	2	4	7	8	10	11	Mean
	V														
0		24	20	30	28	24	28	26	26	28	22	28	26	30	27
1		20	24	28	28	24	24	25	24	26	24	26	28	28	26
2		30	32	26	35	32	28	30	26	24	36	35	20	26	28
3		24	28	30	24	28	24	26	28	28	20	26	26	24	25
4		28	26	26	24	20	28	25	28	24	28	26	24	28	26
5		24	20	28	28	20	24	24	26	24	26	24	28	26	26
6		26	28	24	24	24	24	25	24	28	28	20	26	28	26
7		28	26	28	26	28	26	27	24	24	20	24	26	28	24
8		28	24	24	24	28	28	26	24	32	20	24	28	44	29
9		26	28	28	24	32	32	28	28	24	24	24	24	28	25
10		36	48	36	32	36	32	37	24	28	24	26	24	28	26
11		38	45	52	40	36	40	43	20	32	28	28	28	24	27
12		49	48	48	46	38	48	46	24	24	24	28	32	24	26
13		48	48	44	48	44	44	46	28	28	28	24	28	26	27
14		44	40	40	44	32	36	39	24	20	24	28	32	36	27
15		40	44	48	40	38	40	42	20	24	28	24	28	32	26
16		44	40	40	44	40	40	41	24	28	20	20	24	28	24
17		40	44	44	44	44	40	43	20	24	24	24	20	24	23
18		48	40	44	40	40	44	43	24	28	24	26	24	24	25
19		40	40	40	44	40	40	41	20	24	20	24	28	20	23
20		44	40	44	48	40	40	43	24	24	24	24	24	28	25
21		28	56	40	40	48	52	44	44	40	48	48	40	36	43
22		36	36	44	52	44	36	41	32	44	44	40	32	48	40
23		40	44	36	52	60	36	45	24	36	48	36	28	64	39
24		36	40	36	60	68	32	45	28	36	40	32	36	64	39
25		40	44	44	48	68	32	46	28	32	40	36	32	60	38
26		44	40	36	48	48	40	43	32	36	40	40	40	52	40
27		36	44	44	44	48	36	42	36	40	44	36	32	48	39
28	V	40	40	40	38	52	36	41	32	28	28	28	28	52	33
29		40	28	40	36	64	40	41	28	36	24	28	32	64	35
30		56	32	32	40	64	36	43	32	28	32	28	28	72	37
31		36	36	40	48	64	32	43	28	28	48	36	32	72	41
32		44	32	36	48	72	44	46	28	36	40	28	36	72	40
33		42	34	38	42	64	38	43	28	30	34	30	32	66	37
34		42	32	36	42	62	36	42	28	30	34	28	30	66	36
35		48	32	32	48	60	36	43	28	28	36	24	28	72	36
36		44	28	28	36	72	44	42	24	28	36	28	36	64	36
37		48	32	36	32	68	32	41	24	32	32	32	28	56	34
38		52	32	32	28	68	48	43	28	28	36	24	28	40	31
39		40	32	36	40	76	32	43	28	32	36	24	36	36	32
40		44	32	32	36	72	36	42	24	28	36	28	32	40	31

APPENDIX A, TABLE 6 (Contd.)

Days after Vaccination		Group 1.							Group 2.						
		1	3	5	6	9	12	Mean	2	4	7	8	10	11	Mean
41		40	28	36	36	68	32	40	28	32	32	24	32	36	31
42		56	36	36	36	84	48	49	32	28	36	28	24	48	33
43		48	32	36	32	72	28	41	36	28	48	24	28	48	35
44		56	32	28	32	72	40	43	32	32	40	28	28	44	34
45		60	40	36	32	72	36	46	28	32	32	24	28	32	29
46		52	32	48	40	64	36	45	32	28	28	28	24	48	31
47		56	32	40	36	68	40	45	28	28	36	24	28	44	31
48		52	36	36	32	68	36	43	32	32	32	28	24	40	31
49		48	36	48	32	48	32	41	28	36	28	24	24	48	31
50		44	40	44	28	48	28	39	32	36	28	44	24	56	37
51		48	32	56	28	56	28	41	32	36	28	28	28	44	33
52		40	32	50	28	40	28	36	32	36	26	32	32	40	33
53		48	36	40	42	36	28	38	32	30	26	36	20	40	31
54		40	32	40	40	40	24	36	32	28	24	36	28	36	31
55		44	36	44	32	36	32	37	28	32	24	32	32	32	30
56	Ch.	48	32	36	28	44	28	36	24	32	28	24	24	36	28
57		44	32	40	32	36	32	36	28	36	24	28	24	32	29
58		44	32	36	36	40	28	36	32	28	32	32	20	32	29
59		48	36	40	36	36	32	38	32	28	24	28	28	36	29
60		36	36	44	36	36	36	37	30	28	32	28	24	40	30
61		36	40	40	32	40	40	38	36	32	32	32	28	40	33
62		38	40	44	36	40	36	39	40	32	36	32	36	48	37
63		44	44	44	44	48	36	43	48	40	40	44	40	48	43
64		56	44	52	52	56	40	50	56	48	48	48	52	64	53
65		56	40	48	52	56	36	48	52	48	52	52	52	68	54
66		56	44	52	48	48	44	49	60	52	60	64	56	72	61
67		60	48	56	56	60	48	55	72	56	80	76	64	80	71
68		52	48	68	52	56	56	55	76	64	96	84	72	104	83
69		52	52	84	56	52	52	58	84	72	102	96	80	128	94
70		56	56	76	56	56	56	59	88	76	96	92	88	108	91
71		56	60	80	56	D	60	62	84	88	92	100	96	104	94
72		52	60	80	52		64	62	96	92	88	104	108	112	100
73		48	56	84	48		60	59	92	96	92	100			95
74		52	56	76	48		62	59	88	92	92	96			92
75		48	52	72	44		60	55	84	88	88	92			88
76		40	48	72	44		56	52	88	88	84	96			89
77		40	40	60	40		44	45	92	84	80	100			89
78		36	40	64	36		36	42	96	88	84	104			93
79		36	36	60	32		40	41	100	84	88	108			95
80		31	36	56	36		40	40	96	88	92	100			94
81		28	36	52	32		36	37	108	80	88	112			97
82		36	36	56	32		32	38	100	80	88	108			94
83		36	36	60	28		32	38	96	88	92	116			98
84		40	40	64	36		36	43	80	84	88	112			91
85		36	32	60	32		32	38	76	88	84	100			87
86		32	32	56	28		28	35	80	84	84	96			86
87		K	32	K	28		34	31	K	K	80	108			94

APPENDIX A TABLE 7

Body weights in kilograms of individual suckled calves immunised with Dictol (Group 1) at 3 and 7 weeks of age and their controls (Group 2). Both groups were challenged on week 11.

	Group 1.						Mean	Group 2.						Mean
W	1	3	5	6	9	12	S.E.	2	4	7	8	10	11	S.E.
0	33	34	32	34	35	35	34±0.5	34	28	32	32	34	31	32±0.9
1	36	38	34	38	39	36	37±0.7	35	31	35	34	36	35	34±0.7
2	39	40	37	40	41	38	39±0.6	38	34	37	36	39	38	37±0.7
3	41	43	37	43	45	40	41±1.1	39	36	40	39	43	43	40±1.1
4	44	46	40	45	49	45	45±1.2	41	40	44	43	46	48	44±1.2
5	48	50	46	49	50	52	49±0.8	46	45	47	48	53	51	48±1.3
6	52	51	50	54	54	53	52±0.7	50	50	53	52	57	54	53±1.1
7	56	54	53	59	56	57	56±0.91	53	55	57	55	62	56	56±1.3
8	60	57	55	65	61	61	60±1.4	57	60	60	59	65	58	60±1.1
9	68	63	58	70	65	65	65±1.7	60	65	64	63	69	60	63±1.4
10	69	66	60	75	67	68	67±2.0	65	69	67	65	70	62	66±1.2
11	72	68	63	80	71	72	71±2.3	69	74	70	68	73	64	70±1.5
12	75	75	66	83	75	77	75±2.2	70	80	76	73	74	68	73±1.7
13	77	75	70	86	1	78	77±2.6	73	80	77	74	72	71	74±1.4
14	79	75	74	89	-	80	79±2.7	73	78	75	72	-	-	74±1.3
15	80	77	78	92	-	82	82±2.7	73	77	72	68	-	-	72±1.8

APPENDIX A TABLE 8

Larvae per gram of faeces of individual suckled calves immunised with Dictol (Group 1) at 3 and 7 weeks of age and their controls (Group 2). Both groups were challenged on week 11.

Age	Group 1.						Group 2.						Mean
Weeks	1	3	5	6	9	12	2	4	7	8	10	11	S.E.
0	-	-	-	-	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	-	-	-	-	-	-	
2	-	-	-	-	-	-	-	-	-	-	-	-	
3	-	-	-	-	-	-	-	-	-	-	-	-	
4	-	-	-	-	-	-	-	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-	-	-	-	
6	-	-	-	-	-	-	-	-	-	-	-	-	
7	-	-	-	-	-	-	-	-	-	-	-	-	
8	-	-	-	-	-	-	-	-	-	-	-	-	
9	-	-	-	-	-	-	-	-	-	-	-	-	
10	-	-	-	-	-	-	-	-	-	-	-	-	
11	-	-	-	-	-	-	-	-	-	-	-	-	
12	-	-	-	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	D	-	-	-	-	-	D	D	
14	-	-	-	-		-	-	-	-	-			
15	-	-	50	-		-	1,400	200	100	250			487 ± 306

APPENDIX A TABLE 9

Haemagglutination titres of individual suckled calves immunised with Dictol (Group 1) at 3 and 7 weeks of age and their controls (Group 2). Both groups were challenged on week 11.

Weeks after Vaccination	Group 1.						Mean	Group 2.						Mean
	1	3	5	6	9	12	S.E.	2	4	7	8	10	11	S.E.
0	2	2	2	2	3	2	2.2±0.2	-	-	-	-	-	-	
1	5	1	3	3	4	2	3.0±0.6	-	-	-	-	-	-	
2	2	2	2	1	1	2	1.7±0.2	-	-	-	-	-	-	
3	1	2	2	5	2	2	2.3±0.6	-	-	-	-	-	-	
4	1	1	4	1	2	3	2.0±0.5	-	-	-	-	-	-	
5	2	2	2	2	1	2	1.8±0.2	-	-	-	-	-	-	
6	3	3	2	2	2	2	2.3±0.2	-	-	-	-	-	-	
7	4	4	2	2	3	1	2.7±0.5	-	-	-	-	-	-	
8	8	5	2	4	7	3	4.8±0.9	1	1	1	1	1	1	1.0±0.0
9	10	9	5	9	7	7	7.8±0.7	2	2	2	1	1	1	1.5±0.2
10	10	8	5	5	4	7	6.5±0.9	2	2	4	2	1	1	2.0±0.4
11	7	8	9	4	D	6	6.8±0.9	3	2	4	5	D	D	3.5±0.6
12	7	7	11	5		4	6.8±1.2	5	6	4	5			5.0±0.4

APPENDIX B TABLE 1

Pattern of diarrhoea recorded at weekly intervals from 4 groups of calves grazing on O. ostertagi contaminated pastures during 1973, Groups 2 and 3 were previously immunised with γ-irradiated O. ostertagi L_3. Groups 1 and 4 were controls.

Date	Group 1 114	121	780	Total +	Group 2 109	111	113	Total +
1973								
21/5 to 8/8	O	O	O	O	O	O	O	O
15/8		++	+	3				O
22/8			+	1				O
29/8	+	++		3				O
5/9	+			1	++	++		4
12/9	+++	+		4		+	+	2
19/9	+	+		2				O
27/9	++	++	+	5	++	+++	++	7
3/10	+	+++	+	5	+++	++	++	7
10/10	+	+	++	4	+++	+	++	6
17/10				O	+++		+	4
24/10				O	K		++	2
31/10			++	2				O
7/11				O				O
14/11				O				O

Date	Group 3 107	108	110	Total +	Group 4 116	385	13	Total +
1973								
21/5 to 8/8	O	O	O	O	O	O	O	O
15/8	+	+		1				O
22/8	++			3		++		2
29/8	++			2	++	+		3
5/9				O	+		+	2
12/9				O	++			2
19/9		++	++	4	+++	+		4
27/9				O				O
3/10	+			1				O
10/10			++	2		+++		3
17/10				O				O
24/10		+		1		++		2
31/10	+++	++	+++	8		+		1
7/11	+++	+	+++	7		++		2
14/11		+++		3				O

\+ Soft Faeces

++ Semi-fluid Faeces

+++ Fluid Faeces

APPENDIX B TABLE 2

Individual body weights in kilograms recorded at monthly intervals from 4 groups of calves grazing on O. ostertagi contaminated pastures during 1973. Groups 2 and 3 were previously immunised with γ-irradiated O. ostertagi and Dictol*. Groups 1 and 4 were immunised with Dictol only.

DATE	Group 1			Mean S.E.	Group 2			Mean S.E.
	114	121	780		109	111	113	
1973								
23/4	110	110	95	105±5.0	66	73	70	70±2.0
21/5	136	136	112	128±8.0	91	99	95	95±2.3
21/6	136	130	120	129±4.7	109	100	91	100±5.2
20/7	141	132	123	132±5.2	102	102	97	100±1.7
21/8	140	133	125	133±4.3	100	100	98	99±0.7
21/9	143	136	131	137±3.5	97	98	104	100±2.2
21/10	136	143	144	141±2.5	92	102	105	100±3.9
14/11		153	158	155±2.5	-	110	-	110

DATE	Group 3			Mean S.E.	Group 4			Mean S.E.
	107	108	110		13	116	385	
1973								
23/4	76	66	95	79±8.5		86	84	85±1.0
21/5	92	81	125	101±11.9		109	106	107±1.7
21/6	105	95	136	112±12.3	157	130	114	134±12.5
20/7	107	104	145	119±13.2	155	127	120	134±10.7
21/8	109	108	152	123±14.5	155	132	123	137±9.5
21/9	110	114	155	126±14.4	155		130	142±12.5
21/10	100	98	154	117±18.3	178		137	157±20.5
14/11	93	92	154	113±20.5	190		151	170±19.5

* Dictol = X-irradiated D. viviparus L_3
Allen & Hanburys Ltd., Ware, Herts.

APPENDIX B TABLE 3

Individual plasma pepsinogen levels (m.U. of Tyrosine) recorded at weekly intervals during 1973 from 4 groups of calves grazing on O. ostertagi contaminated pastures. Groups 2 and 3 were previously immunised with γ-irradiated O. ostertagi. Groups 1 and 4 were controls.

	Group 1			Mean		Group 2			Mean		Group 3			Mean		Group 4			Mean	
Date	114	121	780	S.E.		109	111	113	S.E.		107	108	110	S.E.		116	385	13	S.E.	
1973																				
13/4						1700	1200	1400	1433±	145	1500	1600	1300	1467±	88					
20/4						1800	1100	1300	1400±	208	2600	1800	1100	1833±	433					
27/4						2300	900	1600	1600±	404	3200	2100	1300	2200±	551					
3/5						2100	900	1500	1500±	346	2300	2100	1600	2000±	208					
10/5	900	1000	1100	1000±	58	2100	900	1300	1433±	353	2100	2300	1800	2067±	145	1100	1000	1000	1033±	33
17/5	1000	900	1200	1033±	88	2500	700	1200	1467±	536	2400	2400	1800	2200±	200	1200	1000	1100	1100±	58
25/5	900	800	1100	933±	88	1900	600	1200	1233±	376	1300	1500	1400	1400±	58	1000	1100	1200	1100±	58
31/5	900	900	800	867±	33	1400	800	500	900±	265	1400	1200	1300	1300±	58	1100	1100	1400	1200±	100
6/6	700	500	600	600±	58	1100	600	1000	900±	153	900	900	700	833±	67	700	700	1500	967±	267
14/6	500	600	600	567±	33	1800	700	900	1133±	338	1100	700	700	833±	133	700	600	2600	1300±	651
21/6	400	400	300	367±	33	1700	800	1100	1200±	265	1100	600	600	767±	167	400	400	2800	1200±	800
28/6	700	600	800	700±	58	2300	1100	1600	1667±	348	1000	700	700	800±	100	500	700	3100	1433±	835
4/7	800	800	800	800±	0	2300	1200	1400	1633±	338	1200	700	800	900±	153	600	700	3500	1600±	950
11/7	1100	1100	1100	1100±	0	3000	1200	1300	1833±	584	1600	800	1100	1167±	233	900	1000	2800	1567±	617
18/7																				
25/7	1300	1200	1400	1300±	58	2900	1200	2000	2033±	491	2300	1100	1000	1467±	418	1800	1700	3200	2233±	484
2/8	1500	2300	1800	1867±	233	3400	1400	1900	2233±	601	2600	1400	1500	1800±	384	2400	1500	3200	2367±	491
8/8	2200	2900	2900	2667±	233	4400	1700	2300	2800±	818	3300	1300	1800	2133±	601	3700	2500	3300	3167±	353
15/8	2400	3800	2900	3033±	410	4400	1900	2500	2933±	753	3900	1900	2100	2633±	636	5300	4500	3300	4367±	581
22/8	3500	5200	3700	4133±	536	6300	2900	3600	4267±	1037	5500	2600	3200	3767±	884	7300	3000	3700	5667±	1332
29/8	3500	6900	4600	5000±	1002	6000	5100	4000	5033±	578	5200	2300	3300	3600±	850	5700	5600	3000	4767±	884

APPENDIX B, TABLE 3 (CONTD).

Date	Group 1			Mean S.E.	Group 2			Mean S.E.	Group 3			Mean S.E.	Group 4			Mean S.E.
	114	121	780		109	111	113		107	108	110		116	385	13	
1973																
5/9	4400	6300	5500	5400± 551	5600	4200	4900	4900± 404	6100	2500	3600	4067±1065	5000	6300	3500	4933± 809
12/9	5200	7300	6000	6167± 612	5100	4200	5300	4867± 338	6200	2200	4400	4267±1157	4200	5700	3200	4367± 726
19/9	6400	6500	5800	6233± 219	5000	5000	6900	5633± 633	6700	2400	5200	4767±1260		4700	4600	4650± 50
27/9	3700	5700	4800	5067± 318	4400	4800	3900	4700± 153	7500	2400	5000	4967±1472		5300	3600	4450± 850
3/10	5600	5900	6000	5833± 120	4300	5200	8100	5867±1146	8900	3500	8600	7000±1752		6500	4000	5250±1250
10/10	4100	7200	6800	6033± 973	4500	6000	7300	5933± 809	9300	3500	8100	6967±1768		8000	3400	5700±2300
17/10	5200	6400	6800	6133± 481	4400	5000	6700	5367± 689	9000	3200	7000	6400±1701		8100	3200	5650±2450
24/10		5800	5400	5600± 200		4600	6400	5500± 900	8400	3200	5900	5833±1501		6900	3300	5100±1800
1/11		5100	4800	4950± 150		4000		4000±	8000	3700	6900	6200±1290		7100	2300	4700±2400
7/11		4400	3400	3900± 500		2800		2800±	3800	3400	3400	3533± 133		5400	2000	3700±1700

APPENDIX B TABLE 4

Individual trichostrongyle* faecal egg counts (eggs/gm) recorded at weekly intervals from 4 groups of calves grazing on O. ostertagi contaminated pastures during 1973. Groups 2 and 3 were previously immunised with γ-irradiated O. ostertagi. Groups 1 and 4 were controls.

Date	Group 1			Mean	Group 2			Mean
	114	121	780	S.E.	109	111	113	S.E.
1973								
				Up to 1/6/73 All Negative				
6/6	-	-	-		-	50	-	17±16.7
14/6	-	-	-		50	-	50	33±16.7
21/6	-	-	-		-	50	-	17±16.7
28/6	-	-	-		-	150	50	67±44.1
4/7	50	100	-	50±28.9	-	-	-	
11/7	-	50	-	17±16.7	100	400	150	217±92.8
18/7	-	50	-	17±16.7	300	50	-	117±92.8
25/7	50	50	150	83±33.3	-	-	-	
1/8	150	600	150	300±150.0	200	50	50	100±50.0
8/8	100	200	200	167±33.3	150	50	-	67±44.1
15/8	150	350	600	367±130.2	200	100	50	117±44.1
22/8	300	650	400	450±104.1	250	100	50	133±60.1
29/8	1250	500	500	750±250.0	200	-	150	117±60.1
5/9	250	700	150	367±169.1	400	100	300	267±88.2
12/9	5900	900	100	2300±1814.8	250	300	250	267±16.7
19/9	550	850	550	650±100.0	600	450	300	450±86.6
27/9	1300	1450	550	1100±278.4	1200	200	250	550±325.3
3/10	850	1100	600	850±144.3	5450	500	650	2200±1625.6
10/10	450	800	800	683±116.7	5700	300	550	2183±1759.8
17/10	-	-	-		-	-	-	
24/10	-	250	200	225±25.0	-	200	500	350±150.0
31/10	-	550	800	675±125.0	-	200	D	200
7/11	-	450	150	300±150.0	-	50	D	50

Date	Group 3			Mean	Group 4			Mean
	107	108	110	S.E.	116	385	13	S.E.
1973								
				Up to 1/6/73 All Negative				
6/6	-	50	-	17±16.7	-	-	-	
14/6	-	-	-		50	-	-	17±16.7
21/6	-	-	-		-	-	50	17±16.7
28/6	-	-	-		-	-	50	17±16.7
4/7	-	-	-		-	-	-	
11/7	-	-	-		-	-	50	17±16.7
18/7	150	-	-	50±	-	50	-	17±16.7
25/7	50	-	-	17±16.7	-	100	-	33±33.3
1/8	50	-	-	17±16.7	400	300	50	250±104.1
8/8	250	-	-	83±83.0	150	250	-	133±72.6
15/8	350	100	-	150±104.0	850	1100	-	650±332.9

Cont'd...

APPENDIX B, TABLE 4 (CONTD.)

Date	Group 3 107	108	110	Mean S.E.	Group 4 116	385	13	Mean S.E.
1973								
22/8	500	250	200	317±92.8	950	1000	-	650±325.3
29/8	400	350	750	500±125.8	350	900	200	483±212.8
5/9	500	200	500	400±100.0	1150	650	-	600±332.9
12/9	200	500	250	317±92.8	500	400	550	483±44.1
19/9	250	600	650	500±125.8	12100	800	100	4333±3888.6
27/9	350	300	700	450±125.8		900	50	475±425.0
3/10	400	300	350	350±28.9		750	100	425±325.0
10/10	450	200	600	417±116.7		1000	100	550±450.0
17/10	-	-	-		-	-	-	
24/10	250	500	550	433±92.8		1050	-	525±525.0
31/10	1300	1250	1250	1267±16.7		500	100	300±200.0
7/11	2650	800	1300	1583±552.5		1200	50	625±575.0

* Predominantly O. ostertagi, also C. oncophora and T. axei.

APPENDIX B TABLE 5

Individual faecal egg counts (other than trichostrongyles) recorded at weekly intervals from 4 groups of calves grazing on O. ostertagi contaminated pastures during 1973. Groups 2 and 3 were previously immunised with γ-irradiated O. ostertagi and Dictol. Groups 1 and 4 were immunised with Dictol only.

	GROUP 1			GROUP 2		
Date	114	121	780	109	111	113
1973						
	Up to 1/8/73 All Negative					
8/8	1750 E	1000 E	950 E	650 E	550 E	150 E
15/8						
22/8		50 ST			200 E	
29/8						100 E
5/9	200 E	750 E				
12/9			400 E			
19/9	100 D	1000 D	50 D		100 D	100 D
27/9			100 D		100 D	
3/10	250 D	50 D	50 D	150 D		
10/10				600 D		
17/10				100 D		
24/10						
31/10					50 D	

	GROUP 3				GROUP 4	
Date	107	108	110	116	385	13
1973						
	Up to 1/8/73 All Negative					
8/8	450 E	350 E	50 ST	1100 E	950 E	800 E
15/8						
22/8	1450 E	50 ST	250 E			
29/8	1000 E					
5/9						
12/9					300 E	
19/9		150 D		50 D	50 D	600 E
27/9		50 D	50 D	50 D	50 D	
3/10	50 D	50 D			50 D	
10/10	100 E	100 E				
17/10	50 D					
24/10						
31/10						

E Eimeria spp. D D. viviparus

N Nematodirus spp. ST Strongyloides spp.

APPENDIX B TABLE 6

Worm recoveries of Trichostrongylidae other than *O. ostertagi* at autopsy of 4 groups of calves grazing on *O. ostertagi* contaminated pastures during 1973. Groups 2 and 3 were previously immunised with γ-irradiated *O. ostertagi* and Dictol. Groups 1 and 4 were immunised with Dictol only.

Group	Calf No.	*Trichostrongylus axei*		*Cooperia oncophora*				*Nematodirus* spp.	
		Male	Female	Male	Female	L_4	L_5	Male	Female
				VACCINATES					
2	109	1200	2300	1100	1400			100	300
	113			1000	900	5800		200	2600
	111			200	600	300			200
	Mean	400	767	767	967	2033		100	1033
3	107			100	1000	100	100		
	108		100	6100	10200	7600	300	800	700
	110			4500	10600	4000			
	Mean		33	3567	7267	3900	133	267	233
				CONTROLS					
1	114		1200	7800	1800		4400		8600
	121	600		7200	5600	2000			3400
	780			7000	6800	2800			
	Mean	200	400	7333	4733	1600	1467		4000
4	116			1200	1600				
	13			800	800				
	385	1500	1800	6400	7800	6400		800	1000
	Mean	500	600	2800	3400	2133		267	333

APPENDIX B TABLE 7

Analysis of variance comparing O. ostertagi burdens of vaccinated (Groups 2 and 3) with non-vaccinated Controls (Groups 1 and 4).

	ADULT WORMS			
Source of Variation	Degrees Freedom	Sum of Squares	Mean Square	Variance Ratio F
Treatment (Groups 2 & 3 - Vaccinates) (Groups 1 & 4 - Controls)	1	4969470000		1.3942
Replicate	1	2181603333		0.6121
Interaction	1	7291469999		2.0457
Animals Treated Alike	8	28514453333	3564306666	
TOTAL	11	42956996666		

Not Significant

APPENDIX B TABLE 8

O. ostertagi L_3 recovered at fortnightly intervals from naturally contaminated pastures in 1973. Plots 2 and 3 were grazed by calves previously immunised with γ-irradiated *O. ostertagi* and Dictol. Plots 1 and 4 were grazed by control calves vaccinated with Dictol only.

Date	PLOT NO. 1	2	3	4
1973				
Up to 14/5/73	-	-	-	-
28/5	-	124	-	150
11/6	211	240	68	57
25/6	76	250	1758	1370
9/7	1821	420	430	770
23/7	1398	4237	2568	3267
6/8	4661	6432	1428	2885
22/8	8108	18328	1716	10600
5/9	10436	16667	4766	9542
17/9	9316	10641	5634	7247
3/10	11254	12188	3686	11248
19/10	1935	1540	4301	4167
31/10	2097	1015	3574	4391
17/11	1977	3431	2480	8333
28/11	2272	3333	2675	7492
12/12	2143	1818	2778	5172
26/12	1579	1154	2857	5227

APPENDIX B TABLE 9

Trichostrongyloidea L_3 other than *O. ostertagi* recovered at 14 day intervals in 1973 from naturally contaminated pastures. Plots 2 and 3 were grazed by calves previously immunised with γ-irradiated *O. ostertagi* and Dictol. Plots 1 and 4 were grazed by control calves vaccinated with Dictol only.

Date		Plot No. 1	2	3	4
1973					
22/8	C	56	125	172	95
5/9	C	2563	1251	631	236
	D	77	76	-	26
17/9	C	1490	459	716	326
	D	32	26	61	-
3/10	C	1536	1053	655	1425
	D	67	108	88	112
19/10	C	242	1351	345	595
	D	163	67	431	59
31/10	C	242	770	270	286
	D	40	-	50	-
	N	-	250	160	-
17/11	C	565	980	349	192
	D	282	-	500	32
	N	-	-	-	3333
28/11	C	568	833	-	1498
	D	-	-	-	-
	N	-	416	445	749
12/12	C	714	909	694	1293
	D	-	-	-	-
	N	357	303	-	517
26/12	C	789	385	571	1307
	D	-	-	-	-
	N	-	192	571	653

C *Cooperia* spp.

D *Dictyocaulus viviparus*

N *Nematodirus* spp.

APPENDIX B TABLE 10

Pattern of diarrhoea recorded at weekly intervals during 1974 from 2 groups of calves grazing on O. ostertagi contaminated pastures. Calves of Group 1 were previously immunised with γ-irradiated O. ostertagi. Calves of Group 2 were controls.

Date	Group 1. 204	238	885	887	903	Total +	Group 2. 196	881	888	891	Total +
1974											
19/6	+	+	+	+		4	++	++	+		5
26/6	++	++	+	+++	++	10	+++	++	+++	++	10
3/7	++			+	+	4	++	+++	+	+	7
9/7						-	++	++	++		6
17/7				++		2	++				2
24/7			+			1	+		++		3
31/7				+	+	2					-
7/8						-	+	+	+		3
14/8		+	+			2	+				1
21/8						-		+	+		2
28/8	+					1	+	+	+	+	4
4/9						-					
11/9	+					1	+	++			3
18/9		+			+	2	+	+			2
25/9					+	1	+				1
2/10					+	1	+	+		+	4
9/10						-	+		+	++	4
17/10						-			++		2
23/10						-			+	+	2
31/10						-	+		++	++	5

\+ Soft faeces

++ Semi-fluid faeces

+++ Fluid faeces.

APPENDIX B TABLE 11

Individual body weights recorded at monthly intervals during 1974 from 2 groups of calves grazing on O. ostertagi contaminated pastures. Calves of Group 1 were previously immunised with γ-irradiated O. ostertagi and Dictol. Calves of Group 2 were vaccinated with Dictol only.

	Group 1.					Mean	Group 2.				Mean
Date	204	238	885	887	903	S.E.	196	881	888	891	S.E.
1974											
17/4	71	90	47	45	39	58±9.6	73	56	73	55	64±5.1
16/5	81	98	60	55	46	68±9.4	95	65	90	69	80±7.5
24/6	92	111	67	64	60	79±9.8	110	78	105	80	93±8.3
31/7	100	124	80	75	69	90±10.1	125	91	118	93	107±8.6
28/8	109	135	92	88	78	100±10.0	131	96	130	102	115±9.21
25/9	119	145	-	-	86	117±17.1	135	95	137	109	119±10.2
17/10	128	152	-	-	95	125±16.5	138	100	141	115	123±9.8
5/11	139	163	-	-	110	137±15.3	141	103	140	115	125±9.4

APPENDIX B TABLE 12

Individual plasma pepsinogen levels (m.U. of Tyrosine) recorded at weekly intervals during 1974 from 2 groups of calves grazing on *O. ostertagi* contaminated pastures. Calves of Group 1 were previously immunised with γ-irradiated *O. ostertagi* L_3. Calves of Group 2 were controls.

	Group 1.					Mean	Group 2.				Mean
Date	204	238	885	887	903	S.E.	196	881	888	891	S.E.
1974											
18/4	600	400	400	300	400	420± 49	600	500	300	300	425± 75
23/4	800	800	500	300	600	600± 95	800	700	500	500	625± 25
30/4	1200	1000	800	900	900	960± 68	1200	1200	1000	500	975±165
7/5	3200	1300	1800	1100	2600	2000±396	1000	900	900	500	825±111
14/5	1800	1300	1600	1000	2000	1540±178	1000	1100	900	600	900±108
21/5	2100	1200	1500	1600	1600	1600±145	1100	1000	1000	800	975± 63
28/5	1500	800	1100	1300	1200	1180±116	900	800	800	800	825± 25
3/6	1400	800	1000	1000	1200	1080±102	600	700	800	800	725± 48
12/6	1300	900	600	1000	900	940±112	800	900	700	500	725± 85
19/6	1800	800	800	1700	1500	1320±218	1000	1000	800	1000	950± 50
26/6	1200	600	800	900	1100	920±107	900	800	700	700	775± 48
3/7	1600	700	1000	1000	1100	1080/146	1200	1000	900	900	1000± 71
9/7	2000	1100	1000	2600	1400	1620±301	1200	1000	800	1100	1025± 85
17/7	1900	1200	900	1100	1100	1240±172	1400	900	800	900	1000±135
24/7	2500	900	1200	1200	1200	1400±281	2500	1300	1300	800	1475±361
7/8	1500	700	1300	1600	1500	1320±162	1300	1200	1200	1100	1200± 41
14/8	1000	500	900	1100	900	880±102	1300	800	700	800	900±135
21/8	1700	800	1200	1300	1600	1320±159	1200	1000	1100	1000	1050± 50
28/8	1400	1000	600	1200	1300	1100±141	1400	1100	1000	1200	1175± 85
4/9	1900	900	700	1100	1600	1240±223	1700	1200	1400	1200	1375±118
11/9	700	300	700	200	200	420±116	900	500	600	600	650± 87
18/9	2600	1100			2000	1900±308	2800	1700	1800	2400	2175±259
25/9	2000	500			800	1100±458	2800	800	1200	1800	1650±435
2/10	3300	1200			1700	2067±633	3600	2000	2400	3500	2875±399
9/10	2800	1200			1600	1867±481	3100	1700	2700	4600	3025±602
17/10	2700	1400			1600	1900±404	3900	2100	3200	5000	3550±609
23/10	2300	1200			1400	1633±338	3500	1800	2800	4700	3200±610
31/10	2500	1400			1500	1800±351	3600	2200	3800	4200	3450±435
5/11	1600	1000			1200	1267±176	2400	1700	2700	3300	2525±333

APPENDIX B TABLE 13

Individual Trichostrongyle* faecal egg counts (eggs/gm) recorded at weekly intervals during 1974 from 2 groups of calves grazing on O. ostertagi contaminated pastures. Calves of Group 1 were previously immunised with γ-irradiated O. ostertagi and Dictol. Calves of Group 2 were controls.

	Group 1.					Mean	Group 2.				Mean
Date	204	238	885	887	903	S.E.	196	881	888	891	S.E.
1974											
	Up to 26/6 all negative.										
3/7	200	-	100	-	-	60±40.0	50	50	50	200	87±37.5
9/7	150	-	50	-	150	70±33.9	250	-	50	150	112±55.4
17/7	100	-	600	-	-	140±116.6	50	50	200	150	112±37.5
24/7	50	-	50	50	100	50±15.8	-	-	-	-	
31/7	50	50	400	50	-	110±73.1	-	50	-	200	62±47.3
7/8	100	-	250	100	50	100±41.8	50	100	50	250	112±47.3
14/8	200	150	300	250	200	220±25.5	100	50	-	450	150±102.1
21/8	250	-	50	50	50	80±43.6	-	150	50	400	150±89.0
28/8	150	-	250	50	100	110±43.0	-	250	50	150	112±55.4
4/9	-	-	300	50	-	70±58.3	-	-	50	250	75±59.5
11/9	50	-	1050	100	-	240±203.3	100	200	200	650	287±123.1
18/9	50	-	D	D	50	33±16.7	100	150	100	600	237±121.4
25/9	50	50			300	133±83.3	200	150	500	850	425±161.4
2/10	50	50			100	67±16.7	350	250	350	1100	512±197.2
9/10	-	-			-		150	-	600	900	412±206.5
17/10	1	100			-	33±33.3	100	-	1050	100	312±24.7
23/10	50	50			50		-	150	550	150	212±117.9
31/10	50	50			-	33±16.7	150	50	1300	750	562±290.4

* Mainly O. ostertagi, also C. oncophora and T. axei.

APPENDIX B TABLE 14

Individual faecal egg counts other than trichostrongyle recorded at weekly intervals during 1974 from 2 groups of calves grazing on O. ostertagi contaminated pastures. Calves of Group 1 were previously immunised with γ-irradiated O. ostertagi and Dictol. Calves of Group 2 were vaccinated with Dictol only.

	Group 1.					Group 2.			
Date	204	238	885	887	903	196	881	888	891
1974									
3/7	450E							100E	
9/7	50N		50E		50N	1200E			
17/7		600E						250E	100N
24/7				50ST					
31/7				200E			650E		
7/8			300E					50ST	
14/8									
21/8	50ST								50D
28/8					50N	100ST			
4/9									
11/9			150D	200D			50D		
18/9		50D							
25/9									
2/10									
9/10									
17/10									
23/10									
31/10									

E: Eimeria spp. N: Nematodirus spp.

D: D. viviparus ST: Strongyloides spp.

APPENDIX B TABLE 15

Worm recoveries of Trichostrongylidae other than O. ostertagi at autopsy in 1974 of 2 groups of calves grazing on O. ostertagi contaminated pastures. Calves of Group 1 were previously immunised with γ-irradiated O. ostertagi and Dictol. Calves of Group 2 were vaccinated with Dictol only.

Calf Number	Trichostrongylus axei		Cooperia oncophora			Nematodirus spp.	
	Male	Female	Male	Female	L_4	Male	Female
			Group 1				
204			1500	1700	4400	400	1000
238			1700	2200	3600	300	600
885	200	200	3200	2300	100	700	1200
887			100	400	-	100	300
903			1600	1600	2500	700	500
Mean	40	40	1620	1640	2120	440	700
S.E.							
			Group 2				
196			2400	3100	4700	200	500
881			1200	900	1600	200	600
888			4300	3600	7200	300	300
891			900	1200	600	100	700
Mean			2200	2200	3525	200	525

APPENDIX B TABLE 16

O. ostertagi L_3 recovered at fortnightly intervals in 1974 from naturally contaminated pastures. Plots 2 and 3 were grazed alternatively by calves previously immunised with γ-irradiated *O. ostertagi* and Dictol. Plots 1 and 4 were grazed alternatively by control calves vaccinated with Dictol only.

DATE	PLOT NO. 1	2	3	4
1974				
9/1	1923	793	3333	6422
23/1	1555	892	2873	5749
6/2	2500	1388	2115	3750
20/2	2146	2347	1522	3916
6/3	1081	1250	1250	2757
20/3	1672	1333	387	1519
3/4	1093	1631	869	961
17/4	1215	-	751	1244
1/5	2000	714	595	1000
15/5	128	-	120	532
29/5	-	-	-	458
12/6	135	-	-	-
26/6	-	-	-	-
10/7	19	-	-	11
24/7	-	-	-	50
7/8	1412	1006	1029	2575
21/8	4372	2019	2279	3216
4/9	6461	2466	2673	5184
18/9	8910	4769	4433	7218
2/10	7643	4638	4596	7364
16/10	4176	3817	3328	4391
31/10	3254	2247	2638	3578

APPENDIX B TABLE 17

Trichostrongylidae L_3 other than O. ostertagi recovered in 1974 at 14 day intervals from naturally contaminated pastures. Plots 2 and 3 were grazed alternatively by calves previously immunised with γ-irradiated O. ostertagi and Dictol. Plots 1 and 4 were grazed alternatively by control calves vaccinated with Dictol only.

Date		PLOTS 1	2	3	4
1974					
9/1	C	-	793	333	1284
	N	1923	793	333	-
23/1	C	1388	1784	1521	2740
	N	2777	892	-	685
6/2	C	Negative	1388	577	1250
20/2	C	512	1087	Negative	1042
6/3	C	833	-	283	841
	N	-	-	283	-
20/3	C	1431	-	152	325
3/4	C	-	-	-	961
17/4	C	1215	-	-	356
1/5	C	200	-	-	-
15/5	C	32	-	-	-
29/5		-	-	-	-
12/6		-	-	-	-
26/6		-	-	-	-
10/7	N	-	12	14	-
24/7	N	-	-	7	-
7/8	C	180	143	257	322
	N	112	33	10	26
21/8	C	1093	336	325	804
	N	729	288	285	64
4/9	C	1076	411	445	1296
	N	907	247	334	581
18/9	C	1113	935	867	1203
	N	742	477	492	902
2/10	C	955	662	766	920
	N	764	464	511	818
16/10	C	464	636	555	878
	N	418	-	338	399
31/10	C	361	374	440	511
	N	325	225	264	325

C Cooperia spp. N Nematodirus spp.

APPENDIX C TABLE 1

Individual body weights in kilograms from four groups of lambs, three of which were immunised with 2 doses of γ-irradiated H. contortus L_3 (Group 1: 2 x 10,000; Group 2: 2 x 100,000; Group 3: 2 x 1,000,000; Group 4: controls). All groups were challenged with 10,000 normal L_3 4 weeks after the second vaccination.

		Weeks of Age												
Group	Sheep	10	11	12	13	14	15	16	17	18	19	20	21	22
	41	11	13	15	15	15	15	16	17	19	20	21	17	16
	42	10	9	11	12	12	13	13	14	14	16	17	16	15
	44	10	13	14	15	15	17	18	19	19	20	22	19	17
1	45	12	14	15	15	16	16	16	17	17	18	20	20	18
	46	10	11	12	14	14	14	15	16	15	17	18	16	16
	52	11	12	14	14	13	13	13	13	14	15	16	16	16
	55	9	11	12	13	13	14	15	16	16	18	18	18	17
	Mean	10	12	13	14	14	15	15	16	16	18	19	17	16
	S.E.	0.4	0.6	0.6	0.4	0.5	0.6	0.7	0.8	0.8	0.7	0.8	0.6	0.4
	47	13	14	15	16	16	17	19	19	20	22	23	22	20
	48	10	12	12	13	14	14	15	16	16	17	19	18	16
	49	11	9	9	7									
2	51	10	14	11	12	12	13	14	14	15	16	17	16	16
	54	11	12	12	13	13	14	15	15	15	17	18	15	15
	62	10	10	10	11	11	12	13	14	15	16	18	14	14
	64	9	9	8	8	7	7							
	Mean	11	11	11	11	12	13	15	16	16	18	19	17	16
	S.E.	0.5	0.8	0.9	1.2	1.2	1.4	1.0	0.9	1.0	1.1	1.0	1.4	1.0
	50	11	11	11	12	11	11	12	13	13	15	15	13	12
	57	12	13	13	12	11	10	9	9	9				
	58	10	10	10	10	13	9	9						
3	59	11	12	13	13	13	13	15	15	16	17	19	16	14
	61	10	12	12	13	13	13	14	15	15	16	17	16	14
	66	9	7											
	77	10	9	7										
	Mean	10	11	11	12	12	11	12	13	13	16	17	15	13
	S.E.	0.5	0.8	0.9	0.5	0.5	0.8	1.2	1.4	1.5	0.6	1.2	1.0	0.7
	43	12	15	15	14	15	16	16	17	17	20	21	18	17
	53	11	12	14	14	14	15	16	16	17	18	18	16	16
	63	10	12	12	13	13	14	15	15	16	17	19	16	15
4	67	11	12	14	15	13	15	16	18	19	20	22	21	18
	69	12	12	13	14	14	15	16	18	18	19	22	18	17
	70	9	10	10	11	11	12	13	13	14	16	17	17	16
	72	10	12	11	11	12	14	14	15	16	18	18	17	14
	Mean	10	12	13	13	13	14	15	16	17	18	20	18	16
	S.E.	0.4	0.6	0.7	0.6	0.5	0.5	0.5	0.7	0.6	0.6	0.8	0.6	0.5

APPENDIX C TABLE 2

Individual PCV % from four groups of lambs, three of which were immunised with 2 doses of γ-irradiated *H. contortus* L_3 (Group 1: 2 x 10,000; Group 2: 2 x 100,000; Group 3: 2 x 1,000,000; Group 4: controls). All groups were challenged with 10,000 normal L_3 4 weeks after the second vaccination.

		Weeks of Age												
Group	Sheep	10	11	12	13	14	15	16	17	18	19	20	21	22
	41	35	32	30	27	33	32	31	27	30	33	31	20	21
	42	35	31	31	27	28	32	28	28	31	33	28	25	23
	44	30	26	27	23	28	31	26	23	27	27	29	25	23
1	45	38	35	34	35	34	38	36	37	35	35	33	23	23
	46	31	30	28	24	32	34	34	31	34	34	30	21	21
	52	25	29	32	29	31	36	30	30	29	32	27	16	14
	55	38	36	34	27	30	32	29	28	31	31	30	19	21
	Mean	33	31	31	27	31	33	31	29	31	32	30	21	21
	S.E.	1.8	1.3	1.0	1.5	0.9	1.0	1.3	1.6	1.0	1.0	0.7	1.2	1.2
	47	30	25	27	16	28	25	26	26	31	31	33	24	25
	48	30	26	26	15	26	28	24	17	27	31	30	20	22
	49	37	41	36	15	29	D							
2	51	36	32	30	12	25	27	27	29	33	33	28	20	23
	54	37	28	29	20	28	29	33	31	33	35	33	31	28
	62	28	27	28	10	26	32	32	31	34	34	30	19	19
	64	31	31	25	8	20	22	16	D					
	Mean	33	30	29	14	26	27	26	27	32	33	31	23	23
	S.E.	1.4	2.1	1.4	1.5	1.1	1.4	2.5	2.6	1.2	0.8	1.0	2.2	1.5
	50	29	29	27	25	26	34	28	24	26	31	23	13	14
	57	34	32	25	17	30	32	29	26	24	25	D		
	58	33	30	26	20	24	28	20	15	D				
3	59	35	33	24	20	34	33	30	25	32	35	33	24	21
	61	27	24	20	13	23	27	23	20	24	30	26	21	21
	66	34	34	30	18	D								
	71	37	37	23	21	D								
	Mean	33	31	25	19	27	31	26	22	26	30	27	19	19
	S.E.	1.3	1.6	1.2	1.4	2.0	1.4	1.9	2.0	1.9	2.1	3.0	3.3	2.3
	43	36	33	33	32	39	37	34	35	34	33	31	23	22
	53	38	32	35	33	36	34	36	34	35	36	32	23	26
	63	32	30	29	28	30	31	32	31	32	33	29	22	22
4	67	42	36	39	36	29	30	27	30	30	30	27	23	25
	69	39	36	34	35	32	31	32	34	33	33	34	29	28
	70	30	26	26	24	30	34	34	32	29	29	25	18	22
	72	37	36	34	36	34	31	29	31	30	33	28	22	22
	Mean	36	33	33	32	33	33	32	32	32	32	29	23	24
	S.E.	1.6	1.4	1.6	1.7	1.4	0.9	1.2	0.7	0.9	0.9	1.2	1.2	0.9

APPENDIX C TABLE 3

Individual feacel egg counts (eggs/gm) from four groups of lambs, three of which were immunised with 2 doses of X-irradiated *H. contortus* L_3 (Group 1: 2 x 10,000; Group 2: 2 x 100,000; Group 3: 2 x 1,000,000; Group 4: controls). All groups were challenged with 10,000 normal L_3 4 weeks after the second vaccination.

Group	Sheep	Weeks of Age 10	11	12	13	14	15	16	17	18	19	20	21	22
	41	-	-	-	-	-	-	-	-	-	-	-	26000	65200
	42	-	-	-	-	-	-	-	-	-	-	-	4000	25000
	44	-	-	-	-	-	-	-	-	-	-	-	2400	6200
1	45	-	-	-	-	-	-	-	-	-	-	-	23500	42100
	46	-	-	-	-	-	-	-	-	-	-	-	17500	41700
	52	-	-	-	-	-	-	-	-	-	-	-	52200	72400
	55	-	-	-	-	-	-	-	-	-	-	-	23100	21600
	Mean												2143	39171
	S.E.												6279	8988
	47	-	-	-	-	-	-	-	-	-	-	-	22800	27300
	48	-	-	-	-	-	-	-	-	-	-	-	14700	23100
	49	-	-	-	-	-	D							
2	51	-	-	-	-	-	-	-	-	-	-	-	18400	30400
	54	-	-	-	-	-	-	-	-	-	-	-	1800	15500
	62	-	-	-	-	-	-	-	-	-	-	-	30000	24500
	64	-	-	-	-	-	-	-	D					
	Mean												17540	24160
	S.E.												4688	2500
	50	-	-	-	-	-	-	-	-	-	-	-	60000	72800
	57	-	-	-	-	-	-	-	-	-	-	D		
	58	-	-	-	-	-	-	-	-	D				
3	59	-	-	-	-	-	-	-	-	-	-	-	10800	41100
	61	-	-	-	-	-	-	-	-	-	-	-	4600	13000
	66	-	-	-	-	D							25133	42300
	71	-	-	-	-	D							17525	17273
	Mean													
	S.E.													
	43	-	-	-	-	-	-	-	-	-	-	-	22800	15800
	53	-	-	-	-	-	-	-	-	-	-	-	13900	26400
	63	-	-	-	-	-	-	-	-	-	-	-	18100	35100
4	67	-	-	-	-	-	-	-	-	-	-	-	15700	18800
	69	-	-	-	-	-	-	-	-	-	-	-	13100	19500
	70	-	-	-	-	-	-	-	-	-	-	-	45000	28300
	72	-	-	-	-	-	-	-	-	-	-	-	68400	29400
	Mean												28143	24757
	S.E.												7895	2615

APPENDIX C TABLE 4

Individual worm burdens from four groups of lambs, three of which were immunised with 2 doses of γ-irradiated H. contortus L_3 (Group 1: 2 x 10,000; Group 2: 2 x 100,000; Group 3: 2 x 1,000,000; Group 4: Controls). All groups were challenged with 10,000 normal L_3 4 weeks after second vaccination.

Group	Sheep	Total	Male	Female
	41	2,000	1,100	900
	42	2,800	1,800	1,000
	44	1,700	800	900
1	45	4,800	2,400	2,400
	46	4,400	3,600	800
	52	3,300	1,800	1,500
	55	3,000	1,800	1,200
	Mean	3,143	1,900	1,243
	S.E.	433	346	213
	47	3,000	2,000	1,000
	48	3,700	2,100	1,600
	49	DIED BEFORE CHALLENGE		
2	51	2,000	1,400	600
	54	2,400	1,400	1,000
	62	2,700	1,700	1,000
	64	DIED BEFORE CHALLENGE		
	Mean	2,760	1,720	1,040
	S.E.	287	146	160
	50	2,700	2,000	700
	57	2,200	800	1,400*
	58	DIED BEFORE CHALLENGE		
3	59	3,600	2,100	1,500
	61	2,000	1,000	1,000
	66	DIED BEFORE CHALLENGE		
	71	DIED BEFORE CHALLENGE		
	Mean	2,625	1,475	1,150
	S.E.	357	335	185
	43	3,400	2,100	1,300
	53	2,200	1,200	1,000
	63	3,500	2,200	1,300
4	67	3,200	2,000	1,200
	69	2,000	1,600	400
	70	3,600	2,000	1,600
	72	3,200	1,900	1,300
	Mean	3,014	1,857	1,157
	S.E.	243	131	143
5	7	-	-	-
6	8	2,200	1,100	1,100
7	60	200	100	100
8	68	3,200	1,900	1,300

* Died one week after challenge.

APPENDIX C TABLE 5

Individual body weights in kilograms from 3 groups of lambs; Groups 1 (previously exposed to normal larvae) and 2 were immunised with 2 doses of 10,000 γ-irradiated H. contortus L3 and then challenged with 10,000 normal larvae. Group 3 received only the challenge infection.

		Group 1								
Sheep No.		28	29	30	33	39	40	59	Mean	S.E.
Age in Weeks										
10		13	12	15	11	12	12	12	12	0.5
11		14	13	17	10	12	14	13	13	0.8
12		16	15	19	12	12	16	15	15	0.9
13		17	16	20	10	11	14	15	15	1.3
14		17	15	19	12	12	16	15	15	1.-
15		17	17	20	10	13	16	15	15	1.2
16		18	17	21	11	13	16	14	16	1.3
17		18	19	22	14	15	18	9	16	1.6
18		19	19	24	14	15	19		18	1.5
19		21	22	25	15	17	21		20	1.5
20		21	19	24	12	18	18		19	1.6
21		22	22	25	15	23	21		21	1.4
22		23	23	26	16	20	21		21	1.4
23		24	23	26	16	20	22		22	1.4
24		23	22	26	18	21	21		22	1.1
25		25	24	28	18	22	23		23	1.4
26		25	25	28	19	23	24		24	1.2
27		26	24	28	18	24	24		24	1.4
28		26	26	29	19	25	25		25	1.3
29		25	27	28	21	27	25		25	1.-
30		28	29	30	23	28	27		27	1.-
31		29	28	30	26	28	27		28	0.6
32		29	28	30	23	28	27		27	1.-
33		31	30	32	24	29	28		29	1.2
34		32	31	32	24	30	29		30	1.2
35		32	31	32	24	29	29		29	1.2
36		33	33	34	26	31	30		31	1.2
37		34	33	35	25	31	30		31	1.5
38	V	34	33	36	27	32	31		32	1.2
39		34	34	36	28	32	32		33	1.1
40		35	34	38	29	33	32		33	1.2
41		39	34	38	29	34	31		34	1.6
42	V	37	36	42	29	37	34		36	1.7
43		38	36	42	32	37	34		36	1.4
44		37	36	40	31	35	34		35	1.2
45		40	39	43	33	37	36		38	1.4
46	Ch	41	40	43	35	37	38		39	1.2
47		41	41	43	37	39	38		40	0.9
48		44	42	44	38	40	39		41	1.-
49		46	45	48	41	41	41		44	1.3
50		44	43	46	40	42	40		42	1.-

APPENDIX C TABLE 5 (Cont'd.)

Sheep No.		Group 2 31	32	36	38	43	44	52	Mean	S.E.
Age in Weeks										
10		14	12	13	12	11	12	12	12	0.4
11		13	14	14	13	12	13	12	13	0.3
12		14	15	15	14	14	14	14	14	0.2
13		15	18	15	14	15	15	14	15	0.5
14		14	15	16	14	14	14	14	14	0.3
15		16	17	16	15	16	16	16	16	0.2
16		15	17	17	16	17	16	17	16	0.3
17		19	19	18	18	19	18	18	18	0.2
18		21	19	18	19	20	19	18	19	0.4
19		22	22	21	19	21	20	20	21	0.4
20		19	19	19	17	18	17	18	18	0.3
21		24	23	21	20	22	21	21	22	0.5
22		24	24	22	21	23	21	20	22	0.6
23		26	24	21	21	24	22	22	23	0.7
24		27	25	23	23	25	23	22	24	0.7
25		29	26	24	24	26	24	23	25	0.8
26		28	26	24	24	26	23	23	25	0.7
27		28	27	24	25	27	24	23	25	0.7
28		33	28	25	25	28	25	24	27	1.2
29		34	29	24	25	28	25	24	27	1.4
30		36	32	27	29	30	27	22	29	1.7
31		35	31	28	29	29	27	23	29	1.4
32		36	33	26	29	29	29	22	29	1.7
33		39	34	28	30	31	29	18	30	2.4
34		39	35	28	31	32	29	18	30	2.5
35		39	34	27	29	31	29		31	1.8
36		42	37	30	31	34	30		34	1.9
37		45	36	30	32	34	30		34	2.3
38	V	45	38	31	33	35	31		35	2.2
39		46	38	32	33	37	32		36	2.2
40		48	39	31	34	36	33		37	2.5
41		49	39	34	35	39	34		38	2.3
42	V	52	41	34	35	39	36		39	2.7
43		52	43	34	37	42	37		41	2.6
44		54	40	34	36	41	36		40	3.-
45		56	43	37	39	43	37		42	2.9
46	Ch	57	44	38	40	44	37		43	3.-
47		57	44	38	42	46	39		44	2.8
48		58	46	39	43	47	39		45	2.9
49		62	50	40	44	49	39		47	3.5
50		60	46	39	42	46	39		45	3.2

APPENDIX C TABLE 5 (Cont'd.)

Sheep No.		Group 3 35	37	41	46	49	50	58	Mean	S.E.
Age in Weeks										
10		12	15	13	12	11	12	11	12	0.5
11		13	16	13	17	12	13	12	14	0.7
12		11	17	14	18	13	15	14	15	0.9
13		13	17	15	20	14	16	15	16	0.9
14		13	17	16	19	14	16	15	16	0.7
15		15	19	17	21	15	16	17	17	0.8
16		15	20	18	21	16	17	17	18	0.8
17		17	21	20	22	18	20	20	20	0.6
18		18	21	21	23	18	21	21	20	0.7
19		19	25	18	25	18	23	22	21	1.2
20		18	23	23	22	18	21	20	21	0.8
21		19	24	24	26	19	23	24	23	1.-
22		20	25	25	26	20	23	25	23	0.9
23		21	25	25	27	20	24	25	24	0.9
24		21	25	26	27	20	24	25	24	1.-
25		22	26	28	28	21	26	28	26	1.1
26		23	26	29	28	20	26	28	26	1.2
27		23	27	30	29	20	27	28	26	1.3
28		26	28	31	28	21	27	29	27	1.2
29		24	29	33	29	21	28	29	28	1.5
30		27	30	34	32	24	29	31	30	1.3
31		27	31	34	32	23	29	31	30	1.4
32		27	30	35	32	23	29	30	29	1.4
33		29	32	36	33	24	30	32	31	1.4
34		29	32	37	34	24	31	32	31	1.5
35		27	32	37	33	25	30	32	31	1.5
36		30	34	40	35	27	32	35	33	1.6
37		32	35	40	36	27	33	34	34	1.5
38	V	33	35	41	35	30	34	35	35	1.2
39		34	36	42	37	29	34	36	35	1.5
40		36	37	44	37	30	33	37	36	1.6
41		37	36	45	36	31	35	38	37	1.6
42	V	37	38	43	39	31	36	39	38	1.4
43		39	39	45	39	33	37	39	39	1.3
44		38	38	47	38	33	38	39	39	1.6
45		40	41	48	41	34	39	42	41	1.6
46	Ch	41	43	49	43	37	40	43	42	1.4
47		44	42	50	43	37	41	43	43	1.5
48		41	44	48	44	39	42	44	43	1.1
49		45	43	43	45	41	45	48	44	0.8
50		44	45	44	44	41	44	48	44	0.8

APPENDIX C TABLE 6

Individual PCV% from 3 groups of lambs; Groups 1 (Previously exposed to normal larvae) and 2 were immunised with 2 doses of 10,000 γ-irradiated *H. contortus* L3 and then challenged with 10,000 normal larvae. Group 3 received only the challenge infection.

		Group 1								
Sheep No.		28	29	30	33	39	40	59	Mean	S.E.
Age in Weeks										
10		27	36	31	39	29	35	35	33	1.6
11		29	32	31	38	33	32	31	32	1.1
12		29	33	30	38	32	32	32	32	1.1
13		30	33	34	34	30	32	31	32	0.7
14		31	33	33	34	31	34	35	33	0.6
15		30	34	34	33	29	36	32	33	0.9
16		30	32	32	32	25	35	33	31	1.2
17		24	30	30	27	23	29	34	28	1.4
18		28	33	30	32	27	30	-	30	0.9
19		26	32	29	29	26	31		29	1.-
20		31	35	35	33	26	34		32	1.4
21		27	31	31	30	27	31		29	0.8
22		28	34	36	30	28	31		31	1.3
23		30	33	35	34	30	32		32	0.8
24		31	34	31	35	30	36		33	1.-
25		30	34	30	34	27	30		31	1.1
26		28	32	29	34	28	30		30	1.-
27		33	35	31	37	32	34		34	0.9
28		29	32	31	34	30	35		32	0.9
29		28	32	29	34	30	32		31	0.9
30		29	34	31	38	33	31		33	1.3
31		28	33	32	35	31	37		33	1.3
32		27	32	29	38	30	35		32	1.7
33		27	33	31	33	29	31		31	1.-
34		30	32	32	32	31	32		31	0.3
35		30	33	32	40	31	32		33	1.5
36		26	30	29	31	27	33		29	1.1
37		25	27	28	31	23	31		27	1.3
38	V	28	28	30	35	26	33		30	1.4
39		28	29	31	35	26	32		30	1.3
40		28	31	31	31	26	32		30	0.9
41		27	28	30	32	23	29		28	1.2
42	V	30	33	31	33	27	31		31	0.9
43		29	31	31	34	26	31		30	1.1
44		29	34	32	34	27	35		32	1.3
45		29	32	33	33	27	34		31	1.1
46	Ch	29	32	29	32	26	31		30	0.9
47		29	31	30	35	27	30		30	1.1
48		29	31	34	33	26	32		31	1.2
49		30	30	26	33	26	30		29	1.1
50		29	30	24	30	26	30		28	1.-

APPENDIX C TABLE 6 (Cont'd.)

Sheep No.		Group 2 31	32	36	38	43	44	52	Mean	S.E.
Age in Weeks										
10		35	35	32	37	29	31	34	33	1.-
11		35	36	36	33	29	33	32	33	0.9
12		33	31	30	36	29	30	31	31	0.9
13		31	33	33	35	30	31	35	33	0.8
14		33	37	37	34	33	35	39	35	0.9
15		30	34	35	35	36	34	33	34	0.7
16		30	34	31	33	32	32	32	32	0.5
17		29	33	32	33	32	31	35	32	0.7
18		28	33	37	36	35	32	37	34	1.2
19		27	33	33	35	33	33	32	32	0.9
20		28	35	34	37	35	32	35	34	1.1
21		29	33	34	35	33	31	32	32	0.8
22		33	35	35	36	35	32	36	35	0.6
23		33	37	32	35	33	29	34	33	0.9
24		33	40	35	36	33	30	36	35	1.2
25		34	38	35	37	32	30	35	34	1.-
26		33	35	35	38	32	33	34	34	0.7
27		33	34	33	36	32	33	34	34	0.5
28		31	35	31	36	33	32	36	33	0.8
29		33	36	32	37	36	33	36	35	0.7
30		34	36	33	38	34	33	40	35	1.-
31		33	38	34	35	32	32	44	35	1.6
32		30	37	31	38	32	33	40	34	1.5
33		32	35	30	37	38	33	39	35	1.3
34		30	35	33	37	28	36	26	32	1.6
35		33	36	33	37	35	35	D	35	0.7
36		28	35	32	34	32	29		32	1.1
37		29	33	30	33	31	28		31	0.8
38	V	30	36	32	35	31	30		32	1.1
39		31	36	32	34	31	31		32	0.8
40		31	33	32	33	28	27		31	1.1
41		29	26	29	27	26	25		27	0.7
42	V	31	33	34	30	27	31		31	1.-
43		30	33	30	33	28	27		30	1.-
44		31	31	30	35	31	30		31	0.8
45		31	32	30	33	28	34		31	0.9
46	Ch	31	33	31	31	28	29		30	0.7
47		33	32	31	34	27	32		31	1.-
48		33	32	30	31	27	28		30	0.9
49		30	33	31	25	23	29		28	1.5
50		30	30	30	29	26	32		29	0.8

APPENDIX C TABLE 6 (Cont'd.)

Sheep No.		Group 3 35	37	41	46	49	50	58	Mean	S.E.
Age in Weeks										
10		29	28	30	33	34	29	30	30	0.8
11		29	27	31	31	33	29	30	30	0.7
12		35	30	30	33	31	29	30	31	0.8
13		29	30	27	35	31	30	30	30	0.9
14		29	29	33	37	34	37	33	33	1.2
15		28	31	32	37	35	35	34	33	1.1
16		25	29	30	34	32	32	32	31	1.1
17		26	30	32	29	34	29	32	30	1.-
18		30	33	31	31	37	32	32	32	0.9
19		28	28	33	27	32	31	31	30	0.9
20		31	31	30	32	33	32	35	32	0.6
21		30	28	30	34	32	33	32	31	0.8
22		33	31	31	33	31	32	34	32	0.5
23		32	29	31	31	31	33	32	31	0.5
24		31	32	34	33	33	33	38	33	0.8
25		32	33	29	32	33	31	34	32	0.6
26		30	31	31	33	33	31	32	32	0.4
27		30	29	32	31	33	29	33	31	0.7
28		29	28	32	31	33	30	32	31	0.7
29		28	30	34	33	32	33	36	32	1.-
30		31	29	33	33	33	30	35	32	0.8
31		35	30	31	35	31	33	35	33	0.8
32		32	30	35	35	33	30	33	33	0.8
33		30	30	30	31	32	31	33	31	0.4
34		29	30	34	31	33	32	35	32	0.8
35		32	29	32	31	37	34	37	33	1.1
36		29	25	31	36	30	30	38	31	1.7
37		29	24	29	32	32	30	33	30	1.1
38	V	29	26	29	33	36	33	32	31	1.3
39		32	26	33	29	35	30	33	31	1.1
40		29	26	30	32	32	31	32	30	0.8
41		28	28	29	31	31	30	32	30	0.6
42	V	30	26	30	31	32	32	31	30	0.8
43		28	25	30	30	32	32	30	30	0.9
44		29	29	29	29	36	32	31	31	1.-
45		28	27	31	29	32	31	36	31	1.1
46	Ch	28	27	28	27	33	27	32	29	1.-
47		26	27	31	27	33	30	31	29	1.-
48		26	24	26	27	31	30	29	28	0.9
49		22	18	26	27	27	31	31	26	1.8
50		22	16	23	24	27	26	25	23	1.4

APPENDIX C TABLE 7

Individual faecal egg counts (eggs/gm) from three groups of lambs; Groups 1 (previously exposed to normal larvae) and 2 were immunised with 2 doses of 10,000 γ-irradiated *H. contortus* L_3 and then challenged with 10,000 normal larvae. Group 3 received only the challenge infection.

Group	Sheep	Weeks of Age 13	17	21	25	29	33	37	41	45	49	50
1	28	350	1700	2200	50	1650	350	4450	-	-	-	-
	29	500	50	2200	200	2050	50	4600	-	-	-	-
	30	450	-	900	100	300	-	250	-	-	100	10300
	33	2000	1700	150	250	700	-	2800	-	-	-	-
	39	1350	2200	1700	50	1550	850	5000	-	-	-	-
	40	750	1400	2050	50	400	-	-	-	-	-	-
	59	450	250	2450								
	Mean	836	1043	1575	117	1108	208	2850			17	1717
	S.E.	233	346	360	36	300	140	915			17	1717
2	31										650	7200
	32										-	2000
	36										-	-
	38										9150	42000
	43										1400	15700
	44										-	50
	52											
	Mean										1867	11158
	S.E.										1474	6631
3	35										1850	4400
	37										3200	10100
	41										-	3700
	46										-	-
	49										6300	13600
	50										100	1000
	58										-	3100
	Mean										1636	5129
	S.E.										908	1868

Thiabendazole given on weeks 13, 17, 21, 25, 29, 33, 37, 41 and 45 to lambs of Group 1, and on weeks 37, 41 and 45 to animals of Group 2.

APPENDIX C TABLE 8

Individual worm burdens from three goups of lambs; Groups 1 (previously exposed to normal larvae) and 2 were immunised with 2 doses of 10,000 γ-irradiated H. contortus L_3 and then challenged with 10,000 normal larvae. Group 3 received only the challenge infection.

Group	Sheep No.	Total	Male	Female
	28	1,200	500	700
	29	400	200	200
	30	2,000	1,300	700
1	33	200	100	100
	39	100	-	100
	40	400	200	200
	59	DIED BEFORE CHALLENGE		
	Mean	717	383	334
	S.E.	302	196	117
	31	600	100	500
	32	600	200	400
	36	100	-	100
2	38	2,300	1,400	900
	43	1,500	700	800
	44	100	-	100
	52	DIED BEFORE CHALLENGE		
	Mean	867	400	467
	S.E.	355	227	138
	35	800	500	300
	37	3,000	1,500	1,500
	41	3,000	1,200	1,800
3	46	100	-	100
	49	1,300	700	600
	50	200	100	100
	58	1,200	700	500
	Mean	1,371	671	700
	S.E.	454	206	257
4	103	100	-	100
5	200	2,900	1,400	1,500
6	381	200	100	100
7	89	1,900	1,000	900

APPENDIX C TABLE 9

Individual body weights in Kilograms from 4 groups of lambs; Group 1 (previously given normal larvae + anthelmintic); group 2(normal larvae) and group 3 were immunised with 2 doses of 10,000Y- irradiated H. contortus L3 and challenged with 10,000 normal larvae. Group 4 received only the challenge infection.

		Group 1								
Sheep No.		48	63	71	41	53	61	87	Mean	S.E.
Hb Type		A	A	A	AB	AB	AB	AB		
Age in weeks										
10		19	16	20	15	18	16	15	17	0.8
11		21	18	16	15	19	17	14	17	0.9
12		22	18	18	16	19	17	16	18	0.8
13		23	20	18	18	20	19	16	19	0.8
14		26	21	19	20	21	20	19	21	0.9
15		26	22	20	20	22	22	19	22	0.9
16		25	24	21	23	22	23	21	23	0.6
17		27	25	23	23	25	24	23	24	0.6
18		29	26	24	25	23	25	23	25	0.8
19		24	25	24	24	19	24	23	23	0.7
20		24	28	25	26	22	25	24	25	0.7
21		27	29	25	27	24	27	27	27	0.6
22		29	31	27	28	26	28	29	28	0.6
23		30	30	27	29	27	28	29	29	0.5
24		30	30	27	29	27	29	29	29	0.5
25		31	33	29	32	27	28	32	30	0.9
26		31	31	27	31	27	30	31	28	1.4
27		32	33	28	29	28	29	32	30	0.8
28										
29		34	35	30	33	29	32	33	32	0.8
30		32	34	27	31	29	30	32	31	0.9
31		34	35	29	32	29	31	34	32	0.9
32		35	37	30	34	32	36	35	34	0.9
33		34	37	29	33	29	32	33	32	1.1
34	V	34	37	29	33	29	30	34	32	1.1
35		35	39	31	34	30	33		34	1.3
36		37	43	32	35	32	34		35	1.7
37		37	43	33	36	32	34		36	1.6
38	V	39	43	34	36	34	34		37	1.5
39		40	45	34	36	32	34		37	2.0
40		40	46	34	38	33	36		38	1.9
41		41	48	35	39	34	35		39	2.2
42	CH	43	48	35	38	34	37		39	2.2
43		44	49	37	39	36	38		40	2.0
44		45	50	38	39	36	39		41	2.2
45		46	51	38	41	37	41		42	2.2
46		45	50	37	40	36	40		41	2.2

APPENDIX C TABLE 9 (Cont'd)

		Group 2								
Sheep No.		58	70	79	80	47	56	67	89	Mean	S.E.
Hb Type		A	A	A	A	AB	AB	AB	AB		
Age in weeks											
10		21	19	20	19	15	16	16	16	18	0.8
11		22	20	21	18	15	16	16	17	18	0.9
12		21	20	21	20	17	17	17	19	19	0.6
13		23	21	20	22	17	18	19	20	20	0.7
14		25	23	24	24	18	20	20	21	22	0.9
15		27	24	26	26	20	21	22	23	24	0.9
16		27	26	26	28	20	21	23	23	24	1.0
17		31	29	29	30	24	24	25	26	27	1.0
18		29	27	29	30	21	24	26	26	26	1.0
19		31	27	30	29	21	23	25	27	27	1.2
20		30	28	30	30	20	24	26	28	27	1.3
21		34	31	31	28	22	26	28	29	29	1.3
22		35	31	32	29	24	27	28	30	29	1.2
23		36	32	33	34	24	27	29	29	30	1.4
24		36	34	36	36	26	29	30	32	32	1.3
25		37	33	34	35	24	29	29	31	31	1.5
26		38	33	34	36	24	29	29	32	32	1.5
27		36	32	35	35	26	28	29	34	32	1.3
28											
29		38	34	39	39	28	28	33	34	34	1.6
30		39	33	38	38	28	27	32	35	34	1.6
31		39	34	38	38	27	32	31	38	35	1.5
32		40	34	39	39	29	32	34	37	35	1.4
33		40	33	40	38	28	32	34	39	35	1.6
34	V	42	33	40	39	28	33	35	38	36	1.6
35		43	33	41		29	34	34		36	2.2
36		42	33	42		34	35	36		37	1.6
37		43	33	43		32	33	36		37	2.1
38	V	43	34	44		33	36	36		38	1.9
39		44	34	44		34	36	38		38	1.9
40		44	36	46		37	38	39		40	1.7
41		48	38	46		36	38	40		41	2.0
42	CH	51	40	49		38	40	44		44	2.2
43		46	40	49		40	41	41		43	1.5
44		50	42	49		39	41	42		44	1.9
45		51	44	51		39	42	44		45	2.0
46		51	44	50		39	42	45		45	1.9

APPENDIX C TABLE 9 (Cont'd)

		Group 3							
Sheep No.		49	68	77	42	57	62	Mean	S.E.
Hb Type		A	A	A	AB	AB	AB		
Age in weeks									
10		18	16	16	18	17	15	17	0.5
11		18	15	17	19	18	16	17	0.6
12		19	16	18	20	17	17	18	0.6
13		20	18	18	21	19	18	19	0.5
14		23	19	21	23	23	20	21	0.7
15		24	20	22	24	24	20	22	0.8
16		25	20	23	26	24	21	23	0.9
17		26	23	24	28	28	23	25	1.0
18		27	20	24	27	24	21	24	1.2
19		27	22	24	28	26	22	25	1.0
20		29	22	25	29	28	23	26	1.3
21		30	24	28	30	29	24	27	1.1
22		30	24	27	30	28	24	27	1.1
23		31	24	27	29	29	24	27	1.2
24		33	26	29	31	31	25	29	1.3
25		33	26	28	31	30	26	29	1.2
26		34	26	29	31	32	26	30	1.3
27		33	27	29	31	31	27	30	1.0
28									
29		37	29	32	32	34	28	32	1.3
30		34	28	29	34	32	27	31	1.3
31		35	29	32	34	33	29	32	1.0
32		37	30	32	34	34	29	33	1.2
33		37	30	32	34	34	30	33	1.1
34	V	39	31	33	37	35	30	34	1.4
35		34	31	34	38	36	32	34	1.0
36		34	32	34	38	39	34	35	1.1
37		38	31	34	38	38	34	35	1.2
38	V	37	31	36	39	37	34	36	1.1
39		40	32	36	39	40	35	37	1.3
40		41	34	39	40	40	37	38	1.1
41		44	34	41	42	40	39	40	1.4
42	CH	43	35	39	41	42	39	40	1.2
43		47	36	42	43	46	42	43	1.6
44		45	37	42	43	44	40	42	1.2
45		48	38	43	43	46	41	43	1.4
46		47	34	44	43	45	42	42	1.8

APPENDIX C TABLE 9 (Cont'd)

		Group 4							
Sheep No.		54	69	78	44	59	66	Mean	S.E.
Hb Type		A	A	A	AB	AB	AB		
Age in weeks									
10		19	15	17	19	18	16	17	0.7
11		20	15	17	20	19	17	18	0.8
12		21	15	18	21	19	19	19	0.9
13		23	17	20	22	21	19	20	0.9
14		25	18	22	24	24	21	22	1.0
15		27	19	23	25	25	22	23	1.1
16		28	20	24	26	25	23	24	1.1
17		31	21	26	29	29	29	27	1.5
18		29	20	25	25	26	27	25	1.2
19		28	21	27	28	29	24	26	1.2
20		30	21	27	28	30	25	27	1.4
21		28	25	29	29	28	27	28	0.6
22		32	23	29	30	31	27	29	1.3
23		37	24	29	30	32	28	30	1.8
24		34	24	30	32	34	29	30	1.5
25		34	24	31	32	33	29	30	1.5
26		34	25	31	34	34	31	31	1.4
27		34	26	31	34	35	31	32	1.4
28									
29		36	28	32	36	38	34	34	1.5
30		34	26	33	34	34	32	32	1.3
31		38	26	33	36	37	34	34	1.8
32		38	26	34	39	39	36	35	2.0
33		36	28	33	38	38	35	35	1.5
34	V	38	28	35	39	38	37	36	1.7
35		37	29	34	40	40	39	36	1.8
36		39	29	35	40	41	38	37	1.8
37		38	30	35	42	41	38	37	1.5
38	V	38	31	36	44	43	40	39	2.0
39		36	31	38	45	42	40	39	2.0
40		39	33	39	49	45	43	41	2.3
41		40	34	40	51	46	43	42	2.4
42	CH	43	35	39	49	46	46	43	2.1
43		43	34	42	50	47	46	44	2.3
44		44	36	41	51	50	49	45	2.4
45		44	38	42	51	49	47	45	2.0
46		44	37	42	51	48	47	45	2.0

APPENDIX C TABLE 10

Individual PCV% from 4 groups of lambs; Group 1 previously given normal larvae + anthelmintic; group 2(normal larvae)and group 3 were immunised with 2 doses of 10,000 γ- irradiated H. contortus L3 and challenged with 10,000 normal larvae. Group 4 received only the challenge infection.

	Group 1								
Sheep No.	48	63	71	41	53	61	98	Mean	S.E.
Hb Type	A	A	A	AB	AB	AB	AB		
Age in weeks									
10	30	39	36	29	27	37	38	34	1.8
11	34	35	32	29	30	35	34	33	0.9
12	36	32	33	30	33	37	35	34	0.9
13	36	31	31	26	32	36	29	32	1.4
14	31	34	29	26	33	35	35	32	1.3
15	33	30	32	28	33	36	33	32	1.0
16	39	33	35	30	37	39	34	35	1.2
17	37	32	33	28	33	35	32	33	1.1
18	40	35	31	29	35	36	31	34	1.4
19	39	37	35	30	40	38	34	36	1.3
20	36	35	34	30	35	36	34	34	0.8
21	32	32	33	29	32	34	33	32	0.6
22	29	32	33	31	33	35	32	32	0.7
23	30	30	33	32	33	37	36	33	1.0
24	32	31	32	31	31	35	34	32	0.6
25	30	30	32	30	49	28	33	30	0.6
26	30	32	34	30	30	33	33	32	0.6
27	29	30	33	30	31	30	32	31	0.5
28	33	34	35	30	33	36	35	34	0.7
29	31	28	31	27	28	30	30	29	0.6
30	31	29	33	28	32	35	33	32	0.9
31	29	31	31	28	30	31	29	30	0.5
32	31	30	35	29	32	34	31	32	0.8
33	33	29	33	29	29	34	30	31	0.8
34	31	30	31	28	29	34	27	30	0.9
35	29	29	30	24	30	33		29	1.2
36	30	27	28	26	31	33		29	1.1
37	30	26	29	25	27	31		28	1.0
38	27	24	28	20	26	30		26	1.4
39	29	26	31	20	26	31		27	1.7
40	30	28	28	23	28	32		28	1.2
41	32	28	28	21	27	30		28	1.5
42	28	28	31	25	26	33		28	1.2
43	29	28	30	23	29	32		28	1.2
44	30	28	29	24	28	32		28	1.1
45	28	23	30	23	27	31		27	1.4
46	26	20	30	27	23	29		26	1.5

APPENDIX C TABLE 10 (Cont'd)

Sheep No.	58	70	79	80	47	56	67	89	Mean	S.E.
					Group 2					
Hb Type	A	A	A	A	AB	AB	AB	AB		
Age in Weeks										
10	36	37	29	37	34	33	39	33	35	1.1
11	36	32	36	36	30	37	34	29	34	1.1
12	34	30	35	32	32	35	32	31	33	0.7
13	31	31	32	32	29	33	33	30	31	0.5
14	32	29	30	34	28	33	34	29	31	0.9
15	30	28	30	31	29	31	35	30	30	0.7
16	33	29	29	30	32	29	31	30	31	0.5
17	33	32	29	32	32	30	28	29	31	0.7
18	31	28	30	30	24	27	27	28	28	0.8
19	31	28	33	33	22	30	29	29	29	1.2
20	31	27	32	33	25	30	30	30	30	0.9
21	30	27	31	31	28	31	31	29	30	0.6
22	30	27	33	31	29	34	28	30	30	0.8
23	31	29	31	34	28	31	30	30	30	0.6
24	29	28	31	30	27	31	29	28	29	0.5
25	26	26	29	33	25	31	30	27	28	1.0
26	28	27	32	30	27	30	31	26	29	0.8
27	25	26	28	31	26	26	32	26	27	0.9
28	30	29	30	31	28	30	30	27	29	0.5
29	29	26	26	27	24	25	28	26	26	0.6
30	28	26	29	29	27	28	29	25	28	0.5
31	28	26	27	27	27	29	29	24	27	0.6
32	30	31	28	29	27	32	29	25	29	0.8
33	30	33	27	29	24	32	30	26	29	1.1
34	34	32	32	29	31	34	33	29	32	0.7
35	32	32	30		31	33	32		32	0.4
36	31	35	31		31	32	35		32	0.8
37	31	34	31		27	36	30		31	1.3
38	30	34	28		28	31	34		31	1.1
39	30	35	29		30	33	32		31	0.9
40	32	33	31		30	32	32		32	0.4
41	31	32	30		25	29	32		30	1.1
42	32	31	30		29	29	32		30	0.6
43	33	33	30		29	32	35		32	0.9
44	33	31	31		28	33	32		31	0.8
45	31	30	28		28	30	33		30	0.7
46	30	29	28		26	28	26		28	0.7

APPENDIX C TABLE 10 (Cont'd)

	Group 3							
Sheep No.	49	68	77	42	57	62	Mean	S.E.
Hb Type	A	A	A	AB	AB	AB		
Age in weeks								
10	36	37	37	36	32	35	35	0.8
11	34	34	36	36	35	32	34	0.6
12	36	36	35	32	35	31	34	0.9
13	38	33	35	32	33	31	34	1.0
14	38	35	34	32	34	32	34	0.9
15	37	35	36	34	33	34	35	0.6
16	39	37	37	35	37	31	36	1.1
17	40	37	37	39	36	33	37	1.0
18	38	36	33	31	40	32	35	1.5
19	38	37	35	34	40	32	36	1.2
20	36	33	37	32	38	31	34	1.2
21	37	33	33	34	38	30	34	1.2
22	38	31	37	36	38	30	35	1.5
23	38	32	35	34	38	30	34	1.3
24	38	31	36	35	39	31	35	1.4
25	35	32	33	32	36	32	33	0.7
26	35	30	33	35	32	30	32	0.9
27	36	31	32	34	38	26	33	1.7
28	35	31	35	36	38	30	34	1.2
29	36	33	39	35	31	30	34	1.4
30	35	31	33	32	37	32	33	0.9
31	34	32	32	31	33	29	32	0.7
32	35	32	33	33	37	33	34	0.7
33	34	33	33	32	36	31	33	0.7
34	33	34	36	34	34	32	34	0.5
35	36	33	35	33	32	33	34	0.6
36	33	33	33	31	33	30	32	0.5
37	29	30	28	29	30	26	29	0.6
38	27	30	28	28	28	27	28	0.4
39	30	31	30	27	33	28	30	0.9
40	28	30	33	32	35	30	31	1.0
41	27	30	30	30	33	30	30	0.8
42	28	30	31	33	32	29	30	0.8
43	28	32	32	28	33	28	30	1.0
44	30	28	34	30	34	32	31	1.0
45	28	31	35	32	34	32	32	1.0
46	27	31	30	31	30	29	30	0.6

APPENDIX C TABLE 10 (Cont'd)

Sheep No.	54	69	78	Group 4 44	59	66	Mean	S.E.
Hb Type	A	A	A	AB	AB	AB		
Age in weeks								
10	36	39	37	32	34	35	35	1.0
11	36	36	35	33	31	29	33	1.2
12	35	37	35	30	31	32	33	1.1
13	34	38	35	32	31	31	33	1.1
14	36	39	34	32	31	29	33	1.5
15	35	36	36	33	30	31	33	1.1
16	35	36	35	33	33	34	34	0.5
17	37	38	36	32	33	31	34	1.2
18	38	37	35	33	31	32	34	1.1
19	40	36	34	35	32	35	35	1.1
20	36	37	35	33	34	30	34	1.0
21	35	35	36	32	31	32	33	0.8
22	34	35	34	33	34	32	34	0.4
23	33	37	36	33	31	34	34	0.9
24	35	38	38	33	31	31	34	1.3
25	30	36	33	30	30	28	31	1.2
26	32	35	38	34	30	34	34	1.1
27	31	35	36	31	31	30	32	1.0
28	32	35	35	32	31	30	32	0.8
29	33	40	39	33	31	32	35	1.6
30	32	34	34	38	30	32	32	1.0
31	30	35	31	27	30	30	30	1.1
32	32	39	32	31	31	33	33	1.2
33	30	37	36	28	32	32	32	1.4
34	31	35	34	30	30	36	33	1.1
35	31	35	33	30	30	32	32	0.8
36	32	33	34	30	31	30	32	0.7
37	29	34	33	31	33	30	32	0.8
38	30	35	33	32	33	31	32	0.7
39	29	32	30	33	31	31	31	0.9
40	32	30	31	30	30	32	31	0.4
41	31	33	32	33	32	32	32	0.3
42	30	31	30	32	32	31	31	0.4
43	30	30	30	30	30	31	30	0.2
44	29	28	29	29	28	30	29	0.3
45	27	26	26	27	26	28	27	0.3
46	25	24	21	24	25	26	24	0.7

APPENDIX C TABLE 11

Individual faecal egg counts (eggs/gm) from four groups of lambs. Group 1 (previously given normal larvae + anthelmintic); Group 2 (normal larvae) and Group 3 were immunised with 2 doses of 10,000 γ-irradiated H. contortus L_3 and challenged with 10,000 normal larvae. Group 4 received only the challenge infection.

Group	Hb Type	Sheep	Weeks of Age 13	14	15	16	17	18	19	20	21	22	23	24
1	A	48	50				-				-			
	A	63	700				550				100			
	A	71	3100				550				-			
	AB	41	4450				550				100			
	AB	53	300				600				50			
	AB	61	300				250				50			
	AB	87*	2600				1050				350			
	Mean		1643				507				93			
	S.E.		654				123				46			
2	A	58	600	1000	1500	2050	2000	1750	2600	3400	950	2550	2200	2400
	A	70	3150	7200	5500	5800	5750	6650	7450	7000	2700	8350	6700	6450
	A	79	2500	2350	2500	3200	6900	4600	3600	2700	2850	2950	2700	1800
	A	80*	3200	3100	2900	2400	4650	2700	4300	4900	2850	3900	4050	2550
	AB	47	950	3450	5200	5200	8850	3900	750	3300	3250	4800	4850	3200
	AB	56	900	3700	2900	2350	1650	2500	4500	700	1050	3050	3850	900
	AB	67	1700	5200	4100	3300	6450	4500	8700	4900	3750	6900	4500	3500
	AB	89*	1800	2600	1800	1800	800	5450	2600	600	2400	5600	3550	2950
	Mean		1850	3575	3300	3262	4631	4006	4312	3437	2475	4762	4050	2969
	S.E.		359	670	527	524	1018	579	928	769	352	732	489	576

APPENDIX C, TABLE 11 (CONTD.)

Group	Hb Type	Sheep	Weeks of Age 25	26	27	28	29	30	31	32	33	34	38	45	46
1	A	48	250				-				200				650
	A	63	1950				600				1900		50		3200
	A	71	200				-				-				-
	AB	41	1050				50				700				-
	AB	53	1900				200				1600				1200
	AB	61	1500				200				2150				1000
	AB	87*	950				-				800	2900			
	Mean		1114				150				1050				1008
	S.E.		271				82				318				483
2	A	58	4100	3700	4900	1600	3300	1900	800	2150	4150				-
	A	70	11100	7650	11950	4550	6650	8150	5050	4500	7250				100
	A	79	2700	2600	4700	3650	3000	3850	3800	3150	5550				50
	A	80*	4950	5300	8000	5900	5050	6250	8700	8700	10500	11500			
	AB	47	5200	7200	10450	4750	4050	6350	6900	6750	7650				850
	AB	56	1200	1200	5400	2650	3200	3050	800	300	950				300
	AB	67	2800	4700	6500	2850	250	9400	4550	5250	3600				1200
	AB	89*	3450	5250	5500	3850	6150	11400	7900	6550	6800	5550			
	Mean		4437	4700	7175	3725	3956	6293	4812	4669	5806				417
	S.E.		1056	771	963	480	719	1157	1055	966	1033				202

APPENDIX C, TABLE 11 (CONTD.)

Groups 3 and 4 all negative except for weeks 38, 45 and 46.

Group	Hb Type	Sheep	38	45	46
3	A	49	50	100	350
	A	68	-	-	-
	A	77	-	-	200
	AB	42	-	-	100
	AB	57	50	-	850
	AB	62	100	-	-
	Mean		33		250
	S.E.		17		132
4	A	54		200	6300
	A	69		1200	4350
	A	78		550	9600
	AB	44		200	4100
	AB	59		1450	2300
	AB	66		1100	6500
	Mean			783	5525
	S.E.			220	1032

* Killed on week 34 to ascertain the worm population before vaccination.

Thiabendazole given on weeks 13, 17, 21, 25, 29 and 33 to lambs of Group 1 and on week 33 to animals of Groups 2, 3 and 4.

APPENDIX C TABLE 12

Individual worm burdens from four groups of lambs: Group 1 (previously given normal larvae + anthelmintic); Group 2 (normal larvae) and Group 3 were immunised with 2 doses of 10,000 γ-irradiated H. contortus L_3 and challenged with 10,000 normal larvae. Group 4 received only the challenge infection.

Group	Treatment	Sheep	Hb Type	Total	Male	Female
1	1,000-2,000 normal L_3 at monthly intervals + Thiabendazole + 2 x 10,000 γ-irradiated L_3 + Challenge	48	A	450	350	100
		63	A	4450	2500	1950
		71	A	0	0	0
		41	AB	0	0	0
		53	AB	850	350	500
		61	AB	100	50	50
		87*	AB	50	50	0
		Mean		975		
		S.E.		708		
2	1,000-2,000 normal L_3 at monthly intervals + 2 x 10,000 γ-irradiated L_3 + Challenge	58	A	0	0	0
		70	A	50	0	50
		79	A	50	0	50
		80*	A	950	600	350
		47	AB	450	100	350
		56	AB	100	50	50
		67	AB	750	400	350
		89*	AB	950	750	200
		Mean		233		
		S.E.		123		
3	2 x 10,000 γ-irradiated L_3 + Challenge	49	A	250	150	100
		68	A	0	0	0
		77	A	200	0	200
		42	AB	0	0	0
		57	AB	150	100	50
		62	AB	0	0	0
		Mean		100		
		S.E.		47		
4	Challenge	54	A	2350	1300	1050
		69	A	1300	650	650
		78	A	4950	2550	2400
		44	AB	1500	700	800
		59	AB	250	100	150
		66	AB	550	250	300
		Mean		1817		
		S.E.		696		
5	10,000 γ-irradiated L_3	94		150	0	150
6	10,000 Normal L_3	81		1800	750	1050
7	10,000 γ-irradiated L_3	82		200	50	150
8	10,000 Normal L_3	72		1650	750	900

* Animals killed to ascertain the worm burdens of sheep in Groups 1 and 2 before challenge and therefore not considered for the mean.

SOME PROBLEMS ASSOCIATED WITH THE VACCINATION OF RUMINANTS AGAINST HELMINTH INFECTIONS

Summary of a Thesis Submitted for the Degree of Doctor of Philosophy of the University of Glasgow

by

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The study in this thesis was concerned with investigations into 3 separate problems which have arisen in the development of immunisation procedures against 3 separate and serious helminth diseases of cattle and sheep. The comparative efficiency of two techniques used to monitor the number of nematode larvae on herbage was also examined.

In Section 1, the previously unresolved problem concerning the immunisation of young milk-fed calves against the lungworm Dictyocaulus viviparus was investigated. Successful immunisation of weaned calves aged at least 8 weeks with two doses of X-ray attenuated infective larvae has been practised in Britain and Western Europe for some years now and the vaccine is commercially available as Dictol. The fact that immunisation with Dictol is not recommended until the weaned calves are 8 weeks-old has precluded the successful control of lungworm disease in dairy herds where calves are grazed from an early age and in beef herds where calves are suckled for several months.

In the current studies, when Dictol was administered to pail-fed milk calves at 3 and 7 weeks of age the resistance to a subsequent experimental challenge 4 weeks later was excellent compared with non-immunised controls. As judged by the criteria of clinical signs, serological response and post-mortem lungworm burdens the immunity acquired by these

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young calves was comparable to that obtained in calves immunised at 8 and 12 weeks-old.

When the immunisation procedure was repeated in suckled calves aged 3 and 7 weeks the degree of resistance to subsequent challenge was good but inferior to that obtained in pail-fed milk calves. Unfortunately, the situation was complicated by the presence of a Mycoplasma infection in the lungs of these calves, and it is possible that this and/or the blocking effect of maternal antibody may have influenced the result.

Nevertheless, in the absence of any concurrent lung infection these experiments indicate that Dictol immunisation of young calves on a milk diet, whether suckled or pail-fed, appears to be a practical proposition and may result in more widespread and effective control of lungworm disease.

In Section 2, immunisation with γ-irradiated larvae of young calves against the abomasal nematode Ostertagia ostertagi was re-investigated. Previous studies had shown that the acquisition of a solid immunity to this parasite was slow and at best it was hoped that immunisation would limit the infection acquired to a tolerable level. In 1973, parasite-naive calves aged 8 weeks-old were immunised with 2 doses of 100,000 O. ostertagi larvae γ-irradiated at 60 Kr and administered orally at an interval of 4 weeks. When subsequently grazed on pasture with a high level of O. ostertagi infection the calves failed to develop a significant resistance and clinical disease of an intensity similar to the controls developed in the immunised calves. In 1974, the immunisation procedure was repeated when the initial level of

pasture infection was low. Although some immunised calves developed mild ostertagiasis, a moderating effect of the vaccine was apparent in that these calves acquired lower worm burdens than the controls, the clinical disease was milder and the subsequent level of infection on the pasture was lower than in the area grazed by the controls. It is doubtful however if this form of immunisation has any practical value for the control of ostertagiasis.

An interesting feature of both experiments was the apparent suppression of Dictol-induced immunity in calves which had prior exposure to severe ostertagiasis.

In Section 3, the pasture levels of O. ostertagi and D. viviparus larvae were monitored and compared using two techniques, one involving sieving and filtration of the herbage washings, and the other repeated sedimentation of the washings; recovery of both species was approximately similar when over 100 L_3/Kg of herbage were present.

Finally, in Section 4 some factors affecting vaccination of lambs against the stomach worm Haemonchus contortus with larvae attenuated by γ-rays were studied. The results confirmed the inability of parasite-naive young lambs, aged 3 months to develop any immunity to challenge with H. contortus following the administration of 2 doses of attenuated larvae at an interval of 4 weeks. This poor response to immunisation occurred independent of the size of the immunising dose. When older parasite-naive lambs aged 9 to 10 months were also vaccinated with 2 doses of 10,000 larvae previously subjected to γ-irradiation at 60 Kr they

developed a highly significant resistance to an experimental challenge 4 weeks later. If however these older lambs were previously exposed to regular infection with normal larvae from an early age the subsequent response to immunisation was impaired. Repeated anthelmintic therapy during the period of larval exposure appeared to exaggerate the unresponsiveness to subsequent immunisation. These observations on the host/parasite relationship occurred independently of the haemoglobin type of the lambs involved.

The innate unresponsiveness of young lambs plus the superimposed acquired unresponsiveness of lambs exposed to infection with H. contortus in early life makes any immediate prospect of vaccination improbable in areas where the parasite is endemic.