Onderstepoort J. vet. Res. 40 (3), 105-114 (1973)

STUDIES ON THE ENHANCEMENT OF IMMUNITY TO *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

C. M. CAMERON and W. J. P. FULS, Veterinary Research Institute, Onderstepoort

ABSTRACT

CAMERON, C. M. & FULS, W. J. P., 1973. Studies on the enhancement of immunity to *Corynebacterium pseudotuberculosis*. *Onderstepoort J. vet. Res.* 40 (3), 105-114 (1973).

Experiments were undertaken to enhance the immune response of mice, guinea pigs and sheep to *C. pseudotuberculosis* vaccine.

The antibody response of guinea pigs was not increased either by the simultaneous administration of formalin inactivated vaccine and heterologous live vaccine, or by vaccines grown on special media and extracted with ether : ethanol or sodium lauryl sulphate, or by endotoxin.

In mice and sheep the immunity was not enhanced either by the intravenous administration of inactivated vaccines or by the subcutaneous injection of live vaccines prepared from avirulent strains.

In sheep an inactivated vaccine, concentrated tenfold, caused a moderate increase in the antibody titre and there was a marked improvement in their immunity. Such sheep were markedly more resistant to the lethal effects of a subacute infection of living bacteria than those that received standard inactivated vaccine.

It is concluded that concentrated *C. pseudotuberculosis* vaccine does not confer an absolute immunity but it would be of value when used shortly before possible exposure to infection, e.g. shearing.

INTRODUCTION

Cameron, Minnaar, Engelbrecht & Purdom (1972) showed that Merino sheep could be successfully immunized against a subacute infection of *Corynebacterium pseudotuberculosis* by administering two subcutaneous injections of 5,0 ml formalin inactivated vaccine containing 0,5% packed cells precipitated with aluminium phosphate gel. This vaccine also conferred a high degree of immunity to mice (Cameron & Minnaar, 1969).

Moreover, sheep could be partially protected against a chronic infection. Although immunized sheep were capable of destroying the majority of bacteria, or of effectively restricting their multiplication, sufficient numbers survived which eventually gave rise to abscesses.

In this study two possible methods of enhancing the antibody response to *C. pseudotuberculosis* were investigated – artificial stimulation of the immunological system of the host and the preparation of a more antigenic antigen.

The commonest method of stimulating the immunological system artificially is to incorporate adjuvants into the vaccine. Although this proved ineffective in a previous investigation (Cameron *et al.*, 1972), the effect of an oil adjuvant formulated by Thomson, Batty, Thomson, Kerry, Epps & Foster (1969) and of a vaccine prepared from cell walls was studied. The former adjuvant gave excellent results with clostridial antigens (Thomson *et al.*, 1969) while the cell wall vaccine emulsified in oil gave good immunity against tuberculosis to mice (Ribi, Larson, Wicht, List & Goode, 1966).

The immune response of the host can also be stimulated by the injection of live bacteria that exhibit an intracellular parasitism, e.g. *Brucella*, *Mycobacteria* and *Salmonella*. These bacteria provoke a cell response which not only results in a certain degree of non-specific resistance but also enables the host to respond to another antigen more effectively (Mackaness & Blanden, 1967). This response was also studied with respect to *C. pseudotuberculosis*.

Since bacterial endotoxin derived from Gram negative bacteria is known to stimulate the antibody response to protein antigens (Johnson, Gaines & Landy, 1956), its effect on the response to *C. pseudotuberculosis* was tested.

The quality of a bacterial antigen can be influenced by the culture medium on which it is produced (Ekstedt & Yoshida, 1969). The antibody response to *C. pseudotuberculosis* vaccines prepared from bacteria grown on three different media was therefore compared in guinea pigs. An attempt was also made to improve the antigens by using broken bacteria or bacteria whose toxic surface lipid had been removed by extraction.

C. pseudotuberculosis is a facultative intracellular parasite (Jolly, 1965 a & b) and, as pointed out previously, effective immunity may be dependent not only on adequate levels of circulating antibody but also on cellular immune mechanisms (Hard, 1969; Cameron *et al.*, 1972). However, experiments in which hyperimmune rabbit serum was used to protect mice passively proved that this is definitely not the case in these animals (Cameron & Engelbrecht, 1971). In sheep humoral immunity alone may be insufficient and the use of live vaccine to induce a cellular immunity might enhance their immune response (Mackaness & Blanden, 1967). Live vaccines prepared from avirulent strains were therefore administered to mice and sheep and the degree of immunity induced compared with the immunity conferred by killed vaccines prepared from virulent strains.

If, on the other hand, humoral immunity is the only mechanism of immunity the antibody levels obtained by conventional methods of immunization may be inadequate to confer a solid protection. To investigate this possibility killed or live vaccine was administered intravenously to sheep.

In mice good immunity can be induced with as little as 0,2 mg dry bacteria (Cameron & Minnaar, 1969) but it was subsequently found that better protection is obtained when larger doses are used (Cameron & Engelbrecht, 1971). In sheep, however, a fourfold

Received 13 August 1973 - Editor

increase in the vaccine dose did not improve the antibody response (Cameron *et al.*, 1972). The vaccine was therefore concentrated tenfold and evaluated in guinea pigs and sheep.

Materials and Methods

Bacterial strains

C. pseudotuberculosis strain 137B, originally obtained from Prof. H. R. Carne*, was used for preparing inactivated vaccine as well as for challenge of experimental animals. Strain 133A was obtained from Mr. R. F. Jones* while strains 5113C (NH) and 12624 were isolated locally from caseous lymphadenitis in a goat and a sheep respectively.

Preparation of vaccines

Formalin inactivated vaccine (containing aluminium phosphate adjuvant) was prepared from strain 137B grown on CB2 medium (Cameron *et al.*, 1972). Details of the variations in the production procedures used in this study are given in the next section.

Assay of immunity

Mice and sheep were immunized by the general procedures outlined previously (Cameron & Minnaar, 1969; Cameron *et al.*, 1972). Specific variations in the basic procedure are given with the particular experiment under the next heading.

Immunized mice were challenged by injecting live bacteria intravenously 2 weeks after the last injection of vaccine and the cumulative deaths were recorded for 2 weeks (Cameron & Minnaar, 1969). Experimental sheep were exposed to infection as described by Cameron *et al.* (1972).

The antibody response in guinea pigs and sheep was followed by means of an agglutination test (Cameron *et al.*, 1972).

Results

Pathogenicity and cultural characteristics of C. pseudotuberculosis

The pathogenicity to mice of the four strains of *C. pseudotuberculosis* was compared by injecting various quantities of live bacteria intravenously and recording the deaths for 14 days. The procedure used has been described previously (Cameron & Minnaar, 1969) and the results are shown in Table 1.

Table 1 Pathogenicity of *C. pseudotuberculosis* strains for mice

Strain	Dose per mouse	Deaths per 10 mice during 14 days
137B	10^7	10
	10^6	10
	10^5	3
	10^4	3
12624	10^7	10
	10^6	10
	10^5	8
	10^4	3
133A	10^7	10
	10^6	1
	10^5	2
	10^4	0
5113C(NH)	10^7	6
	10^6	0
	10^5	0
	10^4	0

It is clear that strains 133A and 5113C (NH) were at least ten times less virulent than the strains 12624 and 137B. It was subsequently found that mice were not killed by the subcutaneous injection of $1–5 \times 10^7$ live bacteria of strains 133A and 5113C (NH).

These two strains were also less virulent to guinea pigs than 12624 and 137B. Whereas $1–5 \times 10^8$ live bacteria of the virulent strains injected subaneously into guinea pigs were lethal within 4 days similar numbers of bacteria of strains 113A and 5113C (NH) produced occasional local lesions only.

The cultural characteristics of the strains were also investigated. As shown in Table 2, they can be differentiated from one another on their ability to grow as a pellicle on CB2 broth and the production of haemolysis on bovine blood tryptose agar plates.

Table 2 Differentiating cultural features of *C. pseudotuberculosis* strains

Strain	Haemolysis on bovine BTA plates	Pellicle formation on CB2 broth
137B	++	++
12624	++	–
133A	–	–
5113C(NH)	–	–

The antibody response in different hosts

The antibody response to standard aluminium phosphate vaccine was compared in groups of six each Merino and Dorper sheep, Angora and Boer goats, calves, 8 rabbits and 12 guinea pigs. The vaccine was administered in two subcutaneous injections 4 weeks apart. The calves were given 10 ml, sheep and goats 5 ml, rabbits 2 ml and guinea pigs 1 ml.

The antibody titres obtained after administration of *C. pseudotuberculosis* vaccine to the different host animals are shown in Fig. 1. Although the dosages used are not necessarily comparable the agglutination titres obtained in guinea pigs and rabbits were consistently higher than the titres obtained in larger animals. There were no appreciable differences in the titres observed in the two breeds of sheep, the two breeds of goats and the calves.

Influence of altered antigen on the antibody response

Bacteria were extracted either with Ether: Ethanol (EE) as described by Cameron, Minnaar & Purdom (1969) or with sodium lauryl sulphate (SLS). For the latter extraction a 5% (m/v) suspension of bacteria was added to a 2% solution of SLS and stirred for 18 h at 37°C. The bacteria were then concentrated by centrifugation, their density adjusted to 1% packed cells and inactivated by the addition of 0,5% formalin. This vaccine, as well as that prepared from EE treated cells, was precipitated with aluminium phosphate gel to give a final concentration of 0,5% packed cells.

Vaccines used to investigate the influence of the medium on the antigenicity of the organism were prepared from bacteria cultured either in a medium in which the nutrient broth was replaced with Brain Heart Infusion (BHI) (Difco)† or in CB2 medium to which 100 mg/ml L-tyrosine was added after sterilization.

*Department of Veterinary Pathology, University of Sydney, N.S.W., Australia.

†Difco Laboratories, Detroit, Michigan, U.S.A.

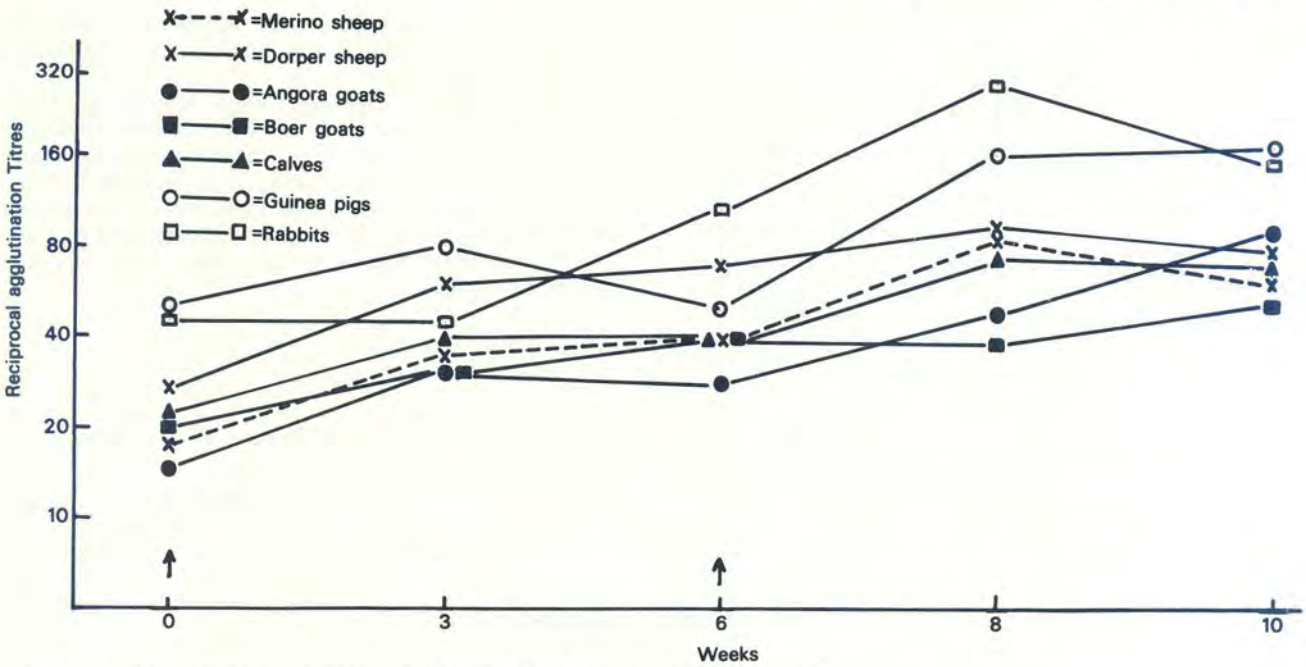

FIG. 1 Antibody response of different host animals to *C. pseudotuberculosis* vaccine

The above four vaccines were assayed in groups of eight guinea pigs. Each guinea pig was given two subcutaneous injections of 1,0 ml each at an interval of four weeks. They were bled at weekly intervals for eight weeks and the antibody titres of the sera were determined by means of an agglutination test.

There was no essential difference between the antibody response stimulated by vaccines prepared from bacteria which were extracted with SLS or EE and vaccine prepared from untreated bacteria (Fig. 2). Moreover there was no difference in the agglutination titres of guinea pigs immunized with vaccines produced in BHI or tyrosine enriched medium as compared with standard vaccine.

Influence of non-specific stimulation on the antibody response

Attempts were made to stimulate the antibody response with live heterologous vaccines and endotoxin using the following products:

Mycobacterium tuberculosis (BCG) (Glaxo); *Brucella abortus* strain 19 (Onderstepoort vaccine); *Salmonella typhimurium* 2656 34 × and *S. dublin* 1/17/5 (Onderstepoort paratyphoid vaccine; and

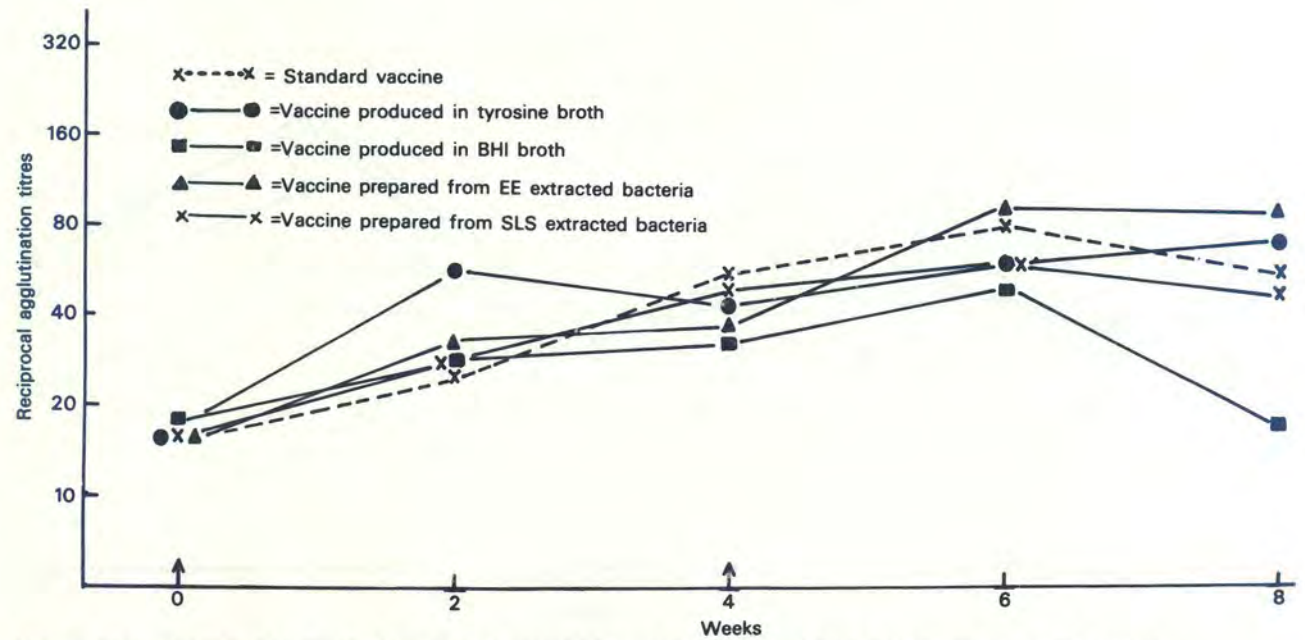

FIG. 2 Antibody response in guinea pigs given *C. pseudotuberculosis* vaccines of which the antigens had been altered.

Escherichia coli endotoxin prepared from serotype 055:B5 (Buxton & Allan, 1963).

Groups of six guinea pigs were given either 1,0 ml BCG, S19 or paratyphoid vaccine which contained 10^6, 10^{10} or 10^8 live bacteria respectively 4 days before or simultaneously with the standard *C. pseudotuberculosis* vaccine. Groups of six guinea pigs were treated with 100 μg *E. coli* endotoxin subcutaneously either 4 days before or simultaneously with *C. pseudotuberculosis* vaccine. The dose of endotoxin (lipopolysaccharide) per guinea pig was based on the findings of Farthing & Holt (1962) who showed that from 10 to 500 μg of *E. coli* lipopolysaccharide enhanced antibody production.

Antibody titres obtained when live BCG, S19, *Salmonella* organisms or *E. coli* endotoxin were given to guinea pigs 4 days before administration of standard *C. pseudotuberculosis* vaccine are shown in Fig. 3a; those obtained when the substances were given simultaneously with the vaccine are illustrated in Fig. 3b. In both instances it is clear that there was no stimulatory effect.

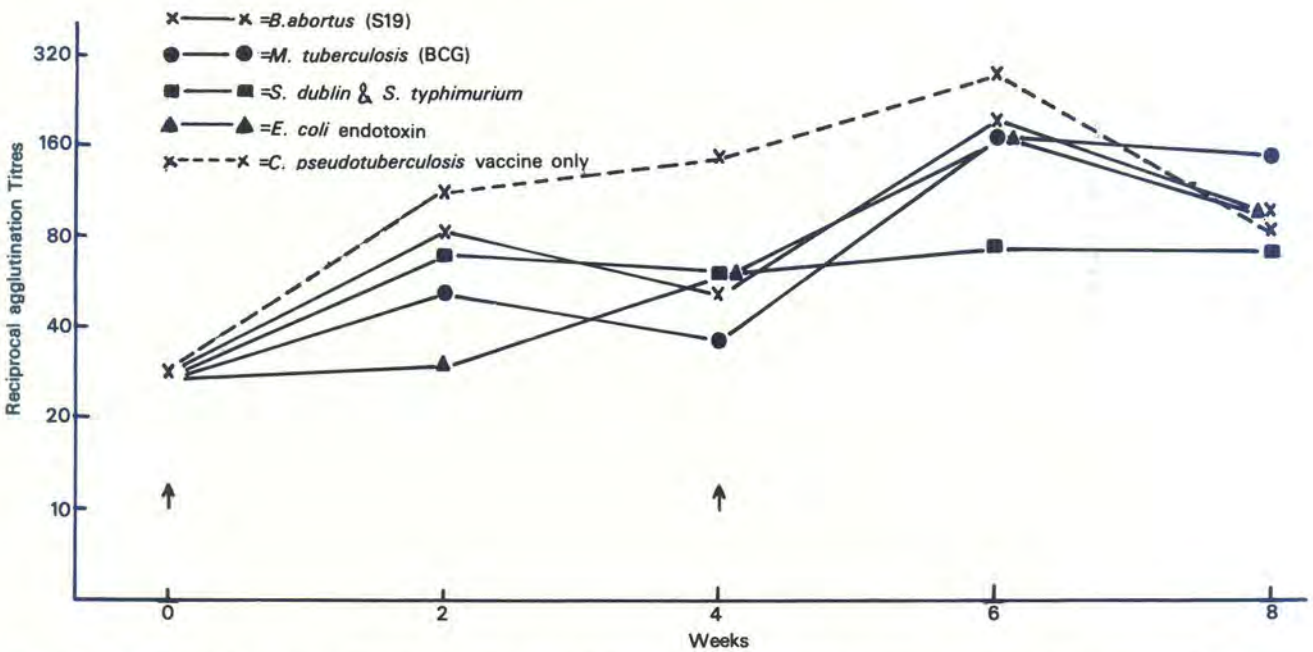

FIG. 3a Effect of live heterologous vaccines and endotoxin given 4 days before killed *C. pseudotuberculosis* vaccine on the antibody response of guinea pigs

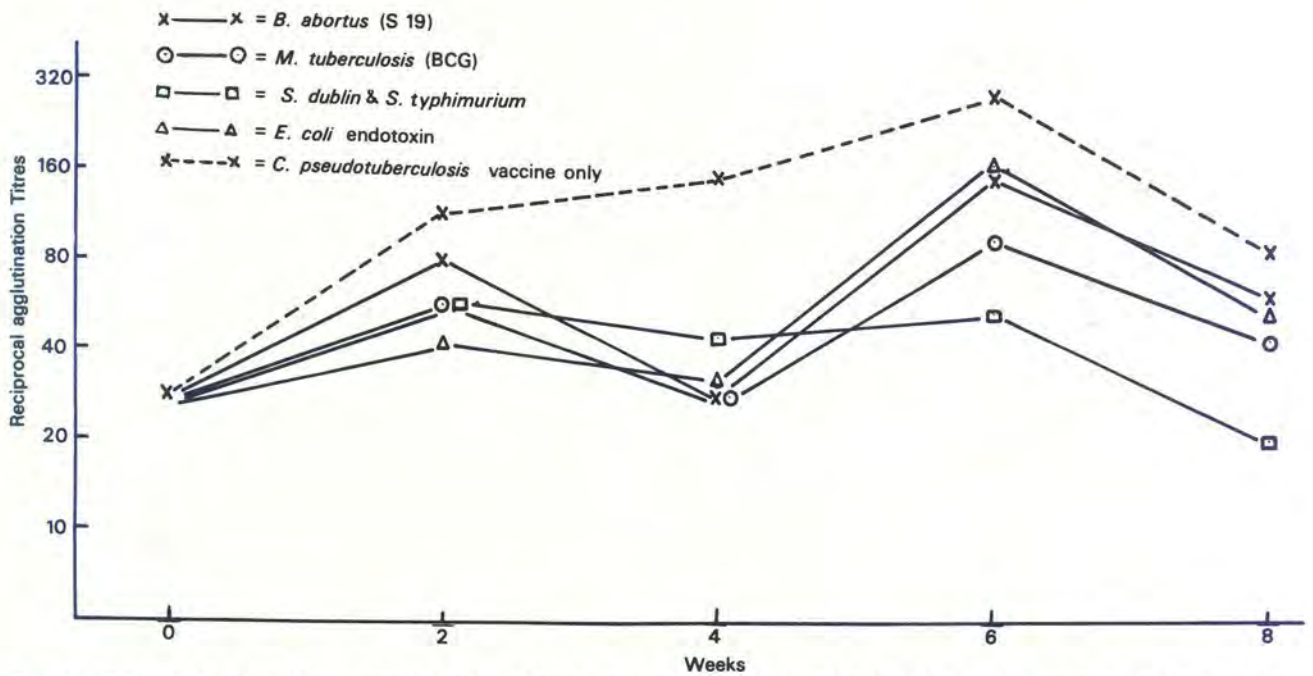

FIG. 3b Effect of live heterologous vaccines and endotoxin given simultaneously with killed *C. pesudotuberculosis* on the antibody response of guinea pigs

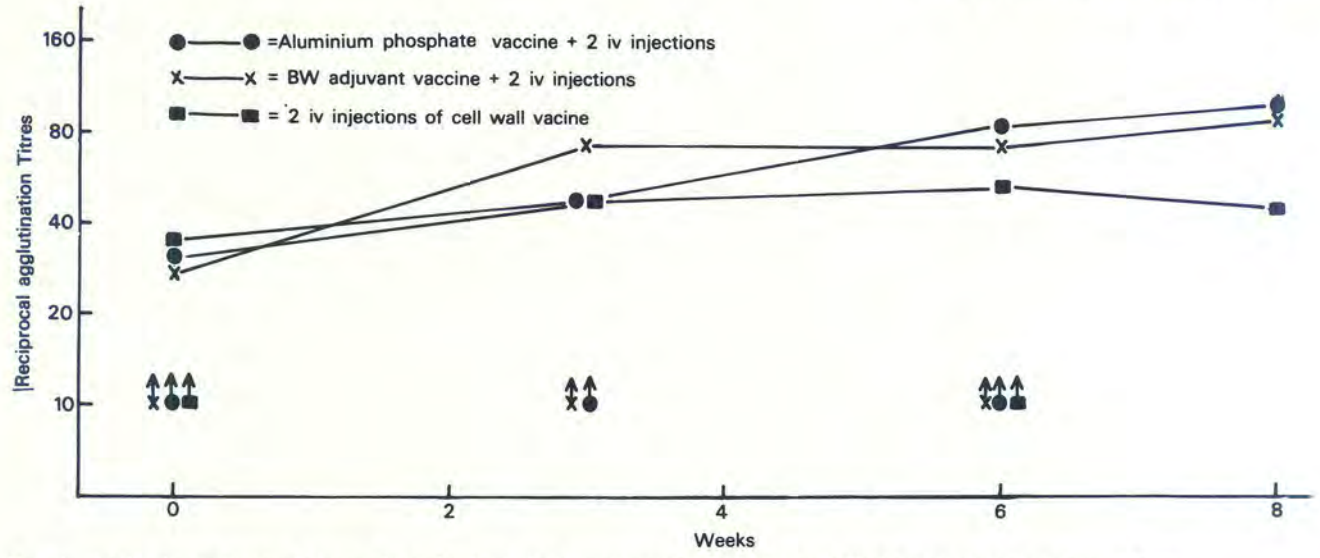

FIG. 4 Antibody response of sheep given dead vaccine intravenously (iv) Arrows indicate when injections were given

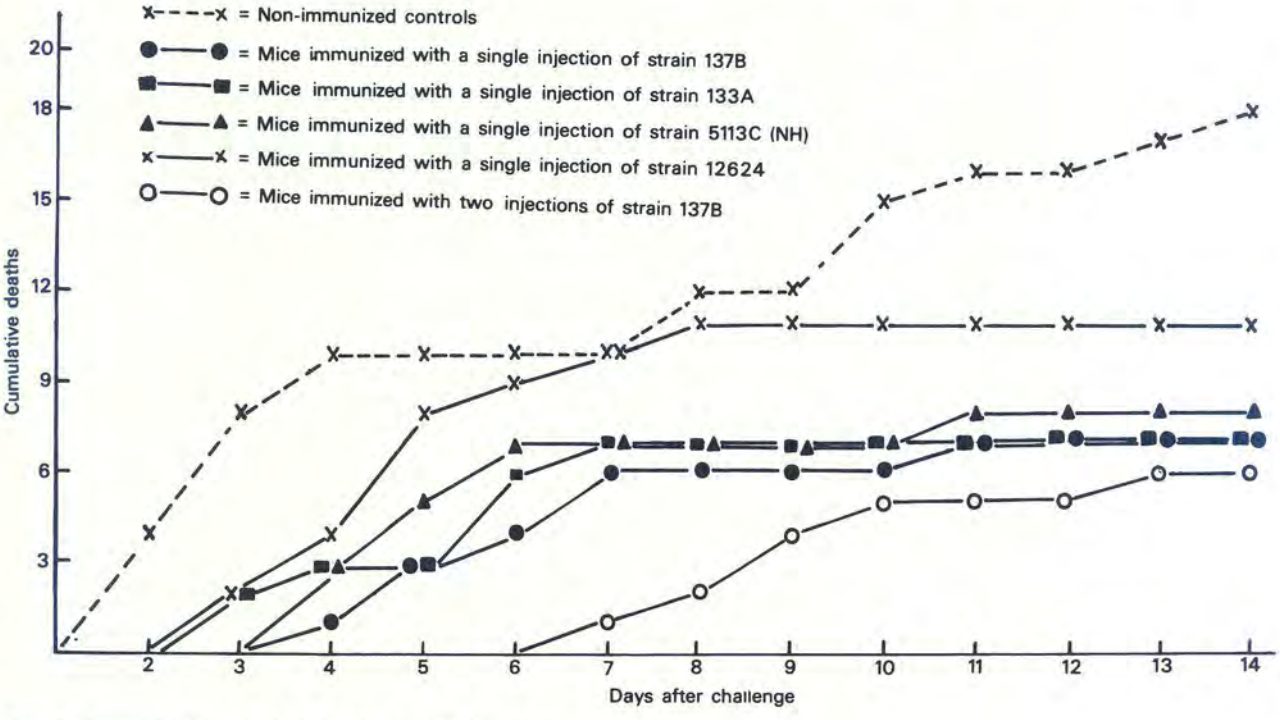

FIG. 5 Immunization of mice with live avirulent bacteria

Immunization with inactivated vaccine given intravenously

Vaccine containing oil adjuvant (oil adjuvant vaccine) was prepared from a 6% suspension of formalin killed bacteria (Thomson *et al.*, 1969). The cell wall vaccine was prepared from dry cell walls as described by Anaker, Ribi, Tarmina, Fadness & Mann (1969) and modified by Cameron *et al.* (1969).

Thirty-two Merino wethers were divided into four groups of eight animals. Group 1 was given an initial subcutaneous injection of 2,0 ml of standard aluminium phosphate vaccine (1,2% packed cells) followed by two intravenous injections of vaccine without adjuvant at intervals of 3 weeks. Group 2 was similarly treated but the first injection consisted of 2,0 ml oil adjuvant vaccine. Group 3 was given two intravenous injections of 2,0 ml cell wall vaccine at an interval of 6 weeks. Group 4 served as controls. Agglutination tests were done at two-weekly intervals and the sheep were challenged 2 weeks after the last injection.

The antibody titres obtained in Groups 1 to 3 of these sheep are shown in Fig. 4. The cell wall vaccine gave almost no response and the antibody titres in the other two groups were lower than the titres which developed after two subcutaneous injections of vaccine.

Unfortunately the exposure of the animals to infection was a failure as the infection rate in the control group was very low and variable.

Immunity produced by live vaccines

Live *C. pseudotuberculosis* vaccine was prepared according to the same procedure used for preparing challenge material for mice (Cameron & Minnaar, 1969). The vaccine was stored at −20°C and the number of live bacteria in aliquots determined by plate counts.

(a) *Mice.* When live vaccine was used the frozen cultures were diluted to give 5×10^7 bacteria per ml. To assess and compare the immunizing potency of live avirulent strains of *C. pseudotuberculosis*, groups of 20 mice were given a single subcutaneous injection of 10^7 live bacteria of the respective strains. At this dosage level strains 12624 and 137B produced local lesions but did not kill the mice while strains 133A and 5113C (NH) caused no detectable reaction.

The mice were challenged 2 weeks later and the cumulative deaths recorded. The results shown in Fig. 5 represent the data from three experiments and demonstrate that although a good immunity is obtained with a single injection of live bacteria, a more solid immunity is produced when two injections are used.

(b) *Guinea pigs.* Thirty-two guinea pigs were divided into four groups of eight animals each. Two groups received a single subcutaneous injection of either 1×10^8 or 5×10^8 live bacteria of strain 5113C (NH) while the remaining groups were treated with two subcutaneous injections of 1 ml of either standard aluminium phosphate vaccine or oil adjuvant vaccine.

Agglutination tests were done at two-weekly intervals and the results are summarized in Table 3. The titres in the groups which received live vaccine were somewhat higher and persisted for a longer period.

(c) *Sheep.* Live homologous vaccines prepared from Strain 133A were tested in three groups of six sheep each. Group A was given a single subcutaneous injection 2 weeks before challenge. Group B received two subcutaneous injections 4 weeks apart while Group C was given one subcutaneous injection followed by two intravenous injections 2 weeks apart. A fourth group of sheep was immunized in the same way as Group C but strain 5113C (NH) was used. The frozen vaccines were diluted to contain 5×10^8 bacteria per ml and a dose of 2,0 ml was used in both cases.

Agglutination titres were determined at two-weekly intervals and the sheep were challenged 2 weeks after the last injection of vaccine.

A good immunity was observed in the groups which received live vaccine intravenously (Fig. 6), and it was associated with a pronounced antibody response (Fig. 7). In the groups which received live vaccine subcutaneously the antibody response was poorer than that obtained with dead vaccine and the resistance to infection was feeble.

Effect of dosage rate

When tenfold concentrated vaccine was required 1,0 l of inactivated culture (1,0% packed cells) was allowed to stand at room temperature for 48 h before 900 ml of the supernatant fluid was removed. The re-

TABLE 3 Agglutinin response of guinea pigs to dead and live *C. pseudotuberculosis* vaccine

Vaccine	Average agglutinin titres				
	Weeks after 1st injection				
	0	2	4	6	8
Aluminium phosphate adjuvant	1:17	1:26	1:32	1:50	1:17
Oil adjuvant	nt	1:25	1:32	1:47	1:14
5113C (NH) live 5×10^8	nt	1:26	1:34	1:62	1:50
5113C (NH) live 1×10^8	nt	1:42	1:84	1:96	1:89

nt = not tested

TABLE 4 Antibody response in sheep given standard and tenfold concentrated vaccine

Groups	Average agglutination titres			
	Weeks after first injection			
	0	3	6	8
Standard vaccine	1:15	1:42	1:26	1:90
Concentrated vaccine	1:15	1:34	1:28	1:145
Control	1:15	1:15	1:15	1:15

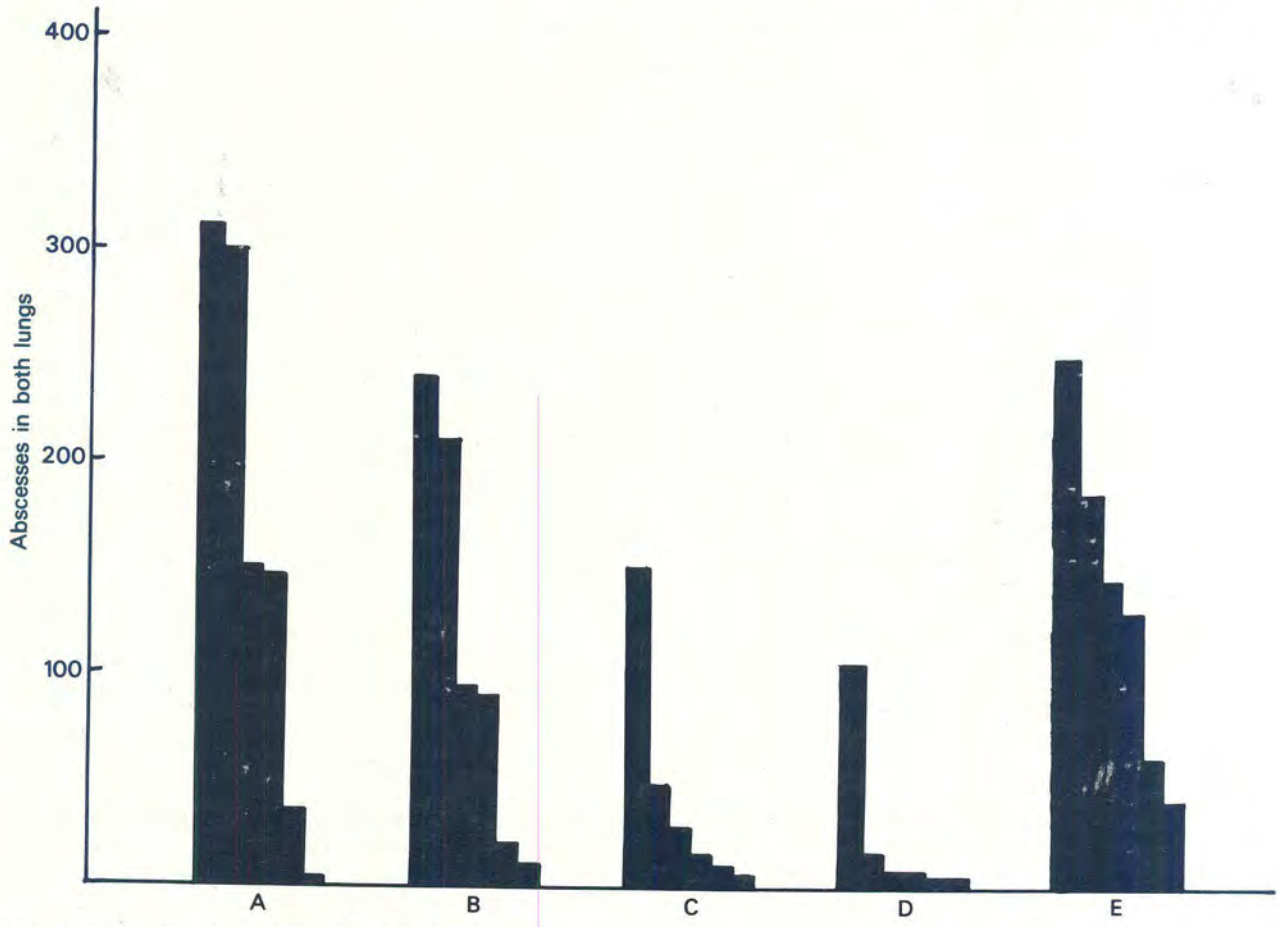

FIG. 6 Immunity of sheep given live vaccines
Group A = Strain 133A —1 subcutaneous injection
Group B = Strain 133A —2 subcutaneous injections
Group C = Strain 133A —1 subcutaneous and 2 intravenous injections
Group D = Strain 5113(NH) —1 subcutaneous and 2 intravenous injections
Group E = Non-immunized controls
Each bar represents the number of abscesses in the lungs of a single individual sheep (6 sheep in a group)

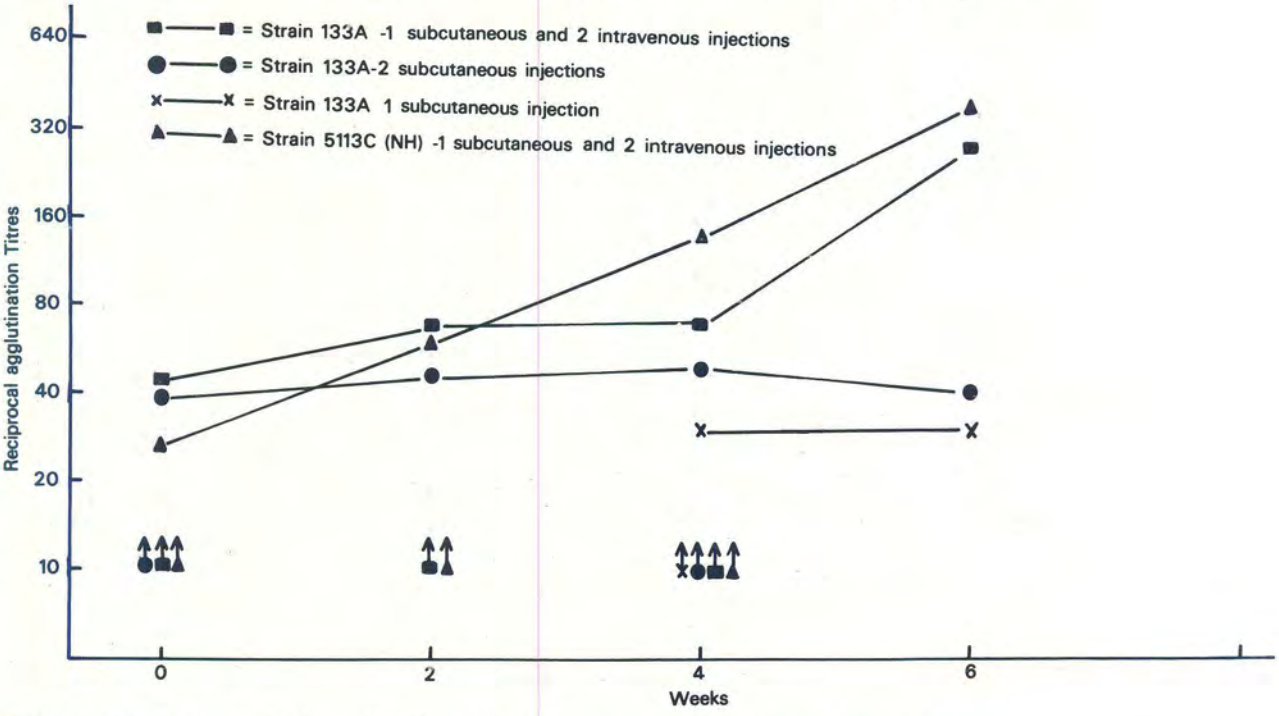

FIG. 7 Antibody response in sheep given live vaccines. The arrows indicate when the injections were given

maining 100 ml was then mixed with an equal volume of aluminium phosphate gel.

(a) *Guinea pigs*. Two groups of 12 guinea pigs were immunized with standard aluminium phosphate adjuvant vaccine and a tenfold concentrated vaccine respectively. In six of the animals from each group the 1,0 ml dose was given at five different sites in 0,2 ml amounts.

The serum agglutination titres of guinea pigs which received standard or tenfold concentrated vaccines were followed for 8 weeks and the results obtained plotted in Fig. 8. As the titres fluctuated considerably, no clear conclusion can be drawn regarding the value of a highly concentrated vaccine.

(b) *Sheep*. Two groups of eight Merino wethers were immunized with two subcutaneous injections of 5,0 ml standard or tenfold concentrated inactivated aluminium phosphate adjuvant vaccine respectively. The two doses of vaccine were given 6 weeks apart and the sheep were challenged 2 weeks after the second injection. The antibody titres are given in Table 4. There was not much difference between the two groups after the first injection but after the second injection the mean titre was notably higher in the group which had received concentrated vaccine.

Unfortunately the challenge dose was too high and the majority of the sheep died within a week. Insufficient animals survived for 4 weeks and therefore autopsies were not carried out to determine the number of abscesses in their lungs.

The cumulative death rates are plotted in Fig. 9. The three groups showed marked differences. Seven of the eight non-immunized control sheep died within 6 days. In the group that received concentrated vaccine the first animal died on the 6th day and only after 24 days had 50% of this group succumbed. The group which received the standard vaccine was more resistant than the control group.

Discussion and Conclusions

Caseous lymphadenitis in sheep is a chronic disease characterized by abscess formation in various lymph nodes and, because of its protracted pathogenesis and the apparent lack of immunity after natural infection, it poses certain unique immunological problems. One important practical complication is the great difficulty with which the natural disease can be simulated. Although a technique has been devised whereby a chronic purulent pneumonia can be established in sheep, the number of experimental animals which can be used is limited. Consequently most of the immunological work on *C. pseudotuberculosis* has been done in mice. While mice can be readily immunized (Cameron & Minnaar, 1969) this is not the case in sheep (Cameron *et al.*, 1972).

In this series of experiments various attempts were made to enhance immunity but all the procedures with non-specific stimulation failed. Similarly, the application of live vaccines did not afford a sound immunity. Although live vaccine given subcutaneously to guinea pigs gave a better antibody response than killed vaccine, this was not the case in sheep. The sheep developed a high antibody titre only when the live bacteria were administered intravenously. These sheep exhibited a good immunity and, as in the case of mice (Cameron & Engelbrecht, 1971) it appears that the humoral antibody level, and not cellular immunity, is the primary mediator of resistance to *C. pseudotuberculosis*.

The poor results following subcutaneous injection of live vaccine are possibly due to the efficiency with which the organisms are sequestered. A thick fibrous capsule rapidly developed around the injected material forming a thick-walled abscess and this effectively prevented the bacteria from coming into contact with the host's immunological system. Encapsulation is not as marked in guinea pigs.

It was, however, found that the dose of vaccine used markedly influenced the degree of immunity which was established. The antibody response in groups of guinea pigs and sheep which received tenfold concentrated inactivated vaccine was not exceptionally high but when the sheep were exposed to infection it was found that they were more resistant. Although

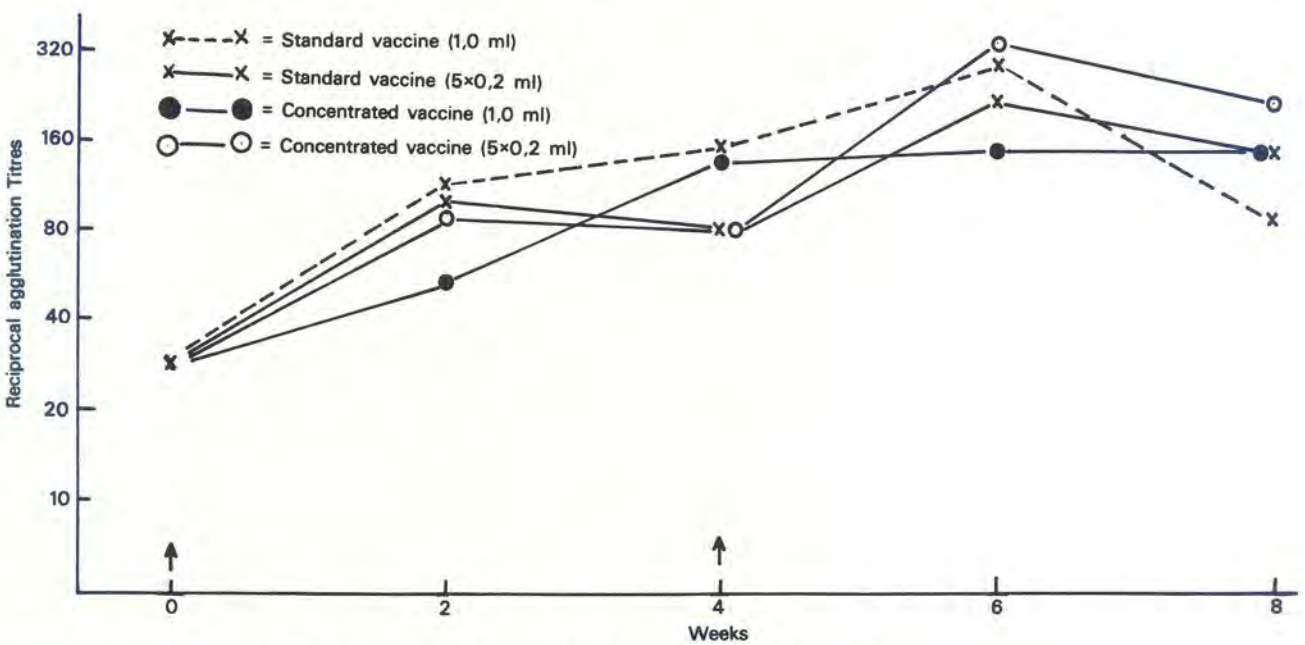

FIG. 8 Antibody response in guinea pigs given standard and tenfold concentration vaccine

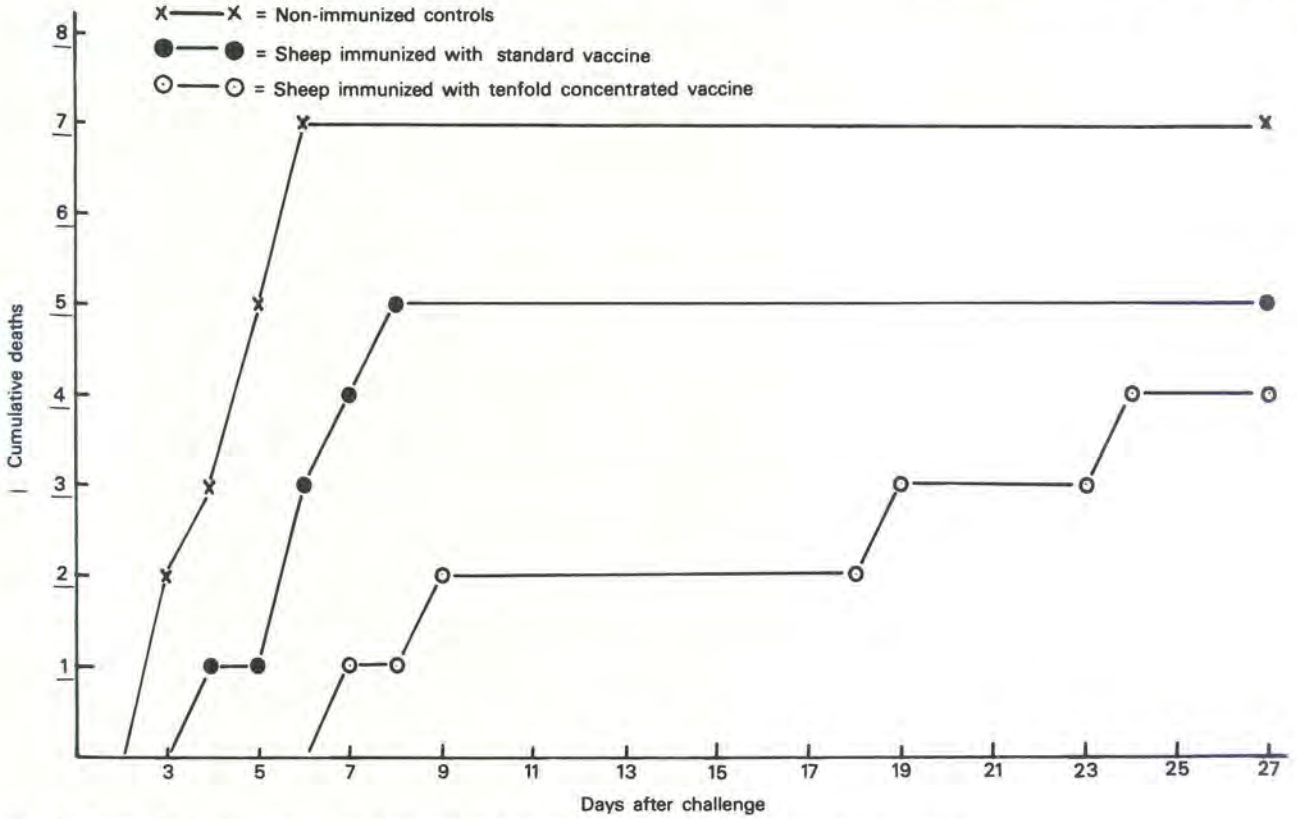

FIG. 9 Immunity in sheep given standard and tenfold concentrated aluminium phosphate vaccine

an absolute immunity could not be established it is important to note that the challenge doses used were vastly in excess of any infection encountered under natural conditions. The concentrated vaccine may therefore prove to be effective when used under field conditions. This can only be proved by extensive and carefully controlled field trials. In the absence of this information and because the antibody response is so brief the suggestion made by Cameron *et al.* (1972) is reiterated, namely that the vaccine be administered shortly before possible exposure to infection, e.g. shearing, so that the animals will have maximum protection during exposure.

ACKNOWLEDGEMENTS

We wish to express our thanks to Miss M. R. Purdom, Mrs. L. van Reenen and Mrs. A. M. Strydom for their technical assistance and to Mr. A. du Bruyn for photographing the graphs.

We also wish to express our particular appreciation to Dr. R. D. Bigalke for his patient advice with respect to the composition of the manuscript.

REFERENCES

ANAKER, R. L., RIBI, E., TARMINA, D. F., FADNESS, L. & MANN, R. E., 1969. Relationship of footpad sensitivity to purified protein derivatives and resistance to airborne infection with *Mycobacterium tuberculosis* of mice vaccinated with mycobacterial cell walls. *J. Bact.*, 100, 51-57.

BUXTON, A. & ALLAN, D., 1963. Studies on immunity and pathogenesis of salmonellosis. I. Antigen-antibody reactions on circulating leucocytes of chickens infected with *Salmonella gallinarum*. *Immunology*, 6, 520-529.

CAMERON, C. M. & Engelbrecht, Maria, M. 1971. Mechanisms of immunity to *Corynebacterium pseudotuberculosis* in mice using inactivated vaccine. *Onderstepoort J. vet. Res.*, 38, 73-82.

CAMERON, C. M. & MINNAAR, J. L., 1969. Immunization of mice against *Corynebacterium pseudotuberculosis*. *Onderstepoort J. vet. Res.*, 36, 207-210.

CAMERON, C. M., MINNAAR, J. L., ENGELBRECHT, MARIA M. & PURDOM, MARY R., 1972. Immune response of Merino sheep to inactivated *Corynebacterium pseudotuberculosis* vaccine. *Onderstepoort J. vet. Res.*, 39, 11-24.

CAMERON, C. M., MINNAAR, J. L. & PURDOM, MARY R., 1969. Immunizing properties of *Corynebacterium pseudotuberculosis* cell walls. *Onderstepoort J. vet. Res.*, 36, 211-216.

EKSTEDT, R. D. & YOSHIDA, K., 1969. Immunity to staphylococcal infection in mice: Effect of living versus killed vaccine, role of circulating antibody, and induction of protection-inducing antigen(s) *in vitro*. *J. Bact.*, 100, 745-750.

FARTHING, J. R. & HOLT, L. B., 1962. Experiments designed to determine the mechanism of the adjuvant activity of Gram-negative organisms upon antibody production. *J. Hyg. Camb.*, 60, 411-426.

HARD, G. C., 1969. Immunity to experimental infection with *Corynebacterium ovis* in the mouse peritoneal cavity. *Res. vet. Sci.*, 10, 547-554.

JOHNSON, A. G., GAINES, S. & LANDY, M., 1956. Studies on the O antigen of *Salmonella typhosa*. V. Enhancement of antibody response to protein antigens by the purified lipopolysaccharide. *J. exp. Med.*, 103, 225-246.

JOLLY, R. D., 1965a. The pathogenesis of experimental *Corynebacterium ovis* in mice. *N.Z. vet. J.*, 13, 141-147.

JOLLY, R. D., 1965b. Experimental infection of convalescent mice with *Corynebacterium ovis*. *N.Z. vet. J.*, 13, 148-153.

MACKANESS, G. B. & BLANDEN, R. V., 1967. Cellular immunity. *In:* Progress in Allergy. Vol. II. Edited by P. Kallos and B. H. Waksman. S. Karger: Basel & New York.

RIBI, E., LARSON, C., WICHT, W., LIST, R. & GOODE, G., 1966. Effective non-living vaccine against experimental tuberculosis in mice. *J. Bact.*, 91, 975-983.

THOMSON, R. O., BATTY, IRENE, THOMSON, A., KERRY, J. B., EPPS, H. B. G. & FOSTER, W. H., 1969. The immunogenicity of a multicomponent clostridial oil emulsion vaccine in sheep. *Vet. Rec.*, 85, 81-85.

Printed in the Republic of South Africa for the Government Printer, Pretoria, by Heer Printing Co. (Pty) Ltd., Pretoria.