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A note on two attenuated strains of myxoma virus isolated in Great Britain

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INTRODUCTION

It has been said that myxomatosis presents a unique opportunity for the study of the effect of a virus disease on a virgin population and it has often been assumed that the Australian course of events would be repeated here. This is not necessarily true as the principal vector is different. Since 1958 very little work has been carried out in Great Britain on the virus strains causing myxomatosis in the field. It was in 1954, approximately 2 years after the initial epizootic, that the first attenuated strain of myxoma virus was isolated in Britain (Hudson, 1954, unpublished; Andrewes, Muirhead-Thomson & Stevenson, 1956; Fenner & Marshall, 1955) in Sussex.

Recently, we have been investigating various aspects of the myxoma virus including an examination of strains isolated from wild rabbits sent to the Central Veterinary Laboratory, Weybridge. This present communication describes the behaviour of two strains isolated in this manner and discusses the implications of the findings in relation to the strains of myxoma virus previously described in this country.

MATERIALS AND METHODS

Virus strains

These are set out in Table 1 together with origin and number of passages.

Rabbits

Domestic rabbits were obtained from Allington Farm, Porton, and a local breeder. Animals were chosen which weighed 2–2½ kg. Rabbits were fed on a diet of pellets and water and were housed in cages in rooms which were heated.

Virus titrations

Virus suspensions were titrated on the chorioallantoic membrane of 11-day-old chick embryos according to the technique of Westwood, Phipps & Boulter (1957).

Virus diluent

This consisted of 200 ml. McIlvaine's citric acid/di-sodium phosphate buffer (Clarke, 1928) at 0·004 M concentration and pH 7·2. To this buffer was added 2 ml.

of skim milk and 0·2 ml. of a penicillin and streptomycin solution which gave a final concentration of 100 units/ml. of penicillin and 100 μg./ml. of streptomycin.

Preparation of virus strains for virulence tests

The material from which the suspensions of different strains of virus were prepared included animal material (Durham, Brecon, KM 13 and Glenfield) and egg pass material (Cornwall and A & H).

The materials containing KM 13, Glenfield, Cornwall and A & H strains were used directly, without pre-treatment, as sources of virus. The materials containing the two field strains (Durham and Brecon) were small pieces of eyelid and lung and were received in 50 % (v/v) glycerol saline. The materials were well washed with cold saline and then ground in a cold pestle and mortar with sterile sand. The ground-up tissues were suspended in the special diluent used in the egg titrations and lightly centrifuged to remove debris and sand. The supernatants containing the virus were stored at −70° C.

Table 1. *Virus strains*

Name	Synonyms and first description	Origin	Number of laboratory passages
Cornwall	England/Cornwall/4-54/1	Diseased wild rabbit	Several
Glenfield	Australia/Dubbo/2-51/1, Fenner & Marshall (1957)	Naturally infected wild rabbit	*c.* 100
KM 13	Aus./Corowa/12-52/2, Fenner & Marshall (1957)	From pool of *Anopheles annulipes*	1
A & H	Andrewes & Harisijades (1955)	Mouse brain adapted (30 passages)	Several (after adaptation
Brecon	4298 (Weybridge 1961)	Diseased wild rabbit	0
Durham	4297 (Webyridge 1961)	Diseased wild rabbit	0

Method of inoculating rabbits

The selected rabbits were inoculated intradermally with a needle contaminated with virus material, in the manner used by Fenner and his co-workers in their more recent experiments (Fenner 1962). Groups of five rabbits were employed for each strain of virus.

Reading of results

The following were recorded in tabular form for each rabbit inoculated:

(1) type of virus;
(2) Date of appearance of primary lesion;
(3) Date of appearance of secondary lesion;
(4) appearance of the primary lesion at the advent of secondaries (*a*) whether flat or raised, and (*b*) lesion colour and whether necrotic;
(5) date of involvement of head and/or eyelids;
(6) date eyes closed;
(7) date of death;
(8) survival time in days.

EXPERIMENTAL RESULTS

Comparison of strains

The two field strains of virus, one from Durham and the other from Brecon, were each inoculated into groups of 5 rabbits and the course of the disease was followed. At the same time known virus strains were inoculated into groups of 5 rabbits for comparison purposes. The results are summarized in Table 2. It became obvious early on that the two field strains were different from the classical virulent English strain (Cornwall), the virulent Australian strain (Glenfield) and the mouse brain adapted strain (A & H). At the same time it was apparent that there was a close similarity between the clinical symptoms produced by the field strains and by the attenuated strain KM 13.

The first part of Table 2 shows a comparison between the times taken for the appearance of the primary and secondary lesions for all the virus strains tested. This table shows that there is no significant difference between the times for the virulent and attenuated strains of virus. However, the very real difference between the primary lesions caused by virulent and attenuated strains of virus is indicated in the last column of Table 2 and shown by Pl. 1, figs. 1*a*, *b*.

It was not possible to distinguish between strains at the advent of the primary lesions. However, as the primaries developed and secondary lesions appeared it became obvious that there was a marked difference between the attenuated and the fully virulent strains. About 6–7 days after inoculation and approximately at the time secondary lesions appeared the primary lesions of the fully virulent strains were raised (about 2–3 cm.) with a somewhat smaller base than the greatest diameter of the lesions, which was of the order of 5 cm. These primary lesions had a very dark red, frequently purple and necrotic centre. In contrast the primary lesions of the attenuated strains were only slightly raised (0·5–1 cm.) and were pink to red in colour. The edges of the lesions were diffuse, the overall diameters were difficult to measure and had a much greater individual variation than those of lesions produced by the fully virulent strains. At 13–15 days most of the rabbits infected with the virulent strains were dead and showed few well-developed secondary lesions. There was, however, involvement of the head with very marked oedema; the eyes were closed and there was a copious purulent discharge from eyes and nose. There was also a discharge from the anus. There were many more secondary lesions at this time in rabbits infected with the attenuated strains. These lesions tended to be much smaller (*c.* 5 mm.) and more nodular. The head and eyelids were not so markedly affected and the discharges from nose, eyes and anus were less than in the rabbits with the virulent strains. The extremities (feet and ears) were affected. There was generally more edema and less localization of reaction in the rabbits given the virulent strains of virus.

Figures 1*a*, *b* show the differences at 15 days. The first series of photographs taken at 6 days were spoilt by the processing laboratory and we did not feel it was justifiable to inoculate another series of rabbits simply for photographs. However a photograph was taken of the 6 day primary lesion of the Brecon strain (Pl. 1, fig. 2), which was the subject of subsequent experiments.

Table 2 also shows the time taken for the production of secondary lesions due

Table 2. *Summary of results*

Virus strain	Those showing disease/ Number of rabbits in a group	Mean; plus range (in days)				Lesion shape and colour at 13 days
		Time taken for appearance of primary lesions	Time taken for appearance of secondary lesions	Time taken for serious head involvement	Mean survival time	
Cornwall (English-virulent)	4/5	4·5; 3–6	7·25; 7–8	9; 8–10	14; 13–15	Raised, necrotic and purple
Glenfield (Australian-virulent)	5/5	2·8; 2–3	6·2; 6–7	7·6; 7–8	15·4; 13–24	Raised, necrotic and dark red
A & H (mouse brain adapted-virulent)	5/5	3·6; 3–6	6·8; 6–7	9; 8–10	15; 13–21	Raised, necrotic and dark red (2 were purple)
KM 13 (Australian-attenuated)	5/5	5; 5	6·8; 6–7	17; 14–20	19·3; 16–22 (with 2 recoveries)	Flat and red
Durham (British field strain)	3/5	5; 3–6	7·66; 7–8	14·5; 14–15	All recovered	Flat and red
Brecon (British field strain) (1)	5/5	5; 5	7; 6–8	13·3; 9–15	29·85; 19–47 (3 recovered)	Flat and red
N	5/5	Not later than 5 days	6; 6			Flat and red
0·05	5/5	Not later than 5 days	5·8; 5–6	12·4; 12–14	21·4; 17–26	Flat and red
6	5/5	Not later than 5 days	5·6; 5–6	Killed at 6 days		

to the Brecon strain when the simple transmission type of inoculation was used (lesion to skin by needle prick) shown under Brecon (1), Brecon N and Brecon 6, and after an intradermal inoculation of 0·05 ml. of the original virus material (Brecon 0·05). The original material contained approximately 1×10^3 p.f.u./ml. so that a 0·05 ml. quantity gave 50 p.f.u. The quantity of virus which is normally transmitted by an insect bite has been calculated to be somewhere in the region of 2 pock forming units (or 5 rabbit infectious doses) (Fenner & Marshall, 1957; Mykytowycz, 1956).

By an examination of the time taken for the serious involvement of the head (closing of the eyes, severe discharge from eyes and nose) (Table 2) it is immediately apparent that there is a considerable difference between the virulent and attenuated strains of virus in this respect.

Examination of one of the field strains

Having deduced, from the comparison of survival times and clinical symptoms of all the virus strains examined, that the field strains (Brecon and Durham) were attenuated, a further examination of the Brecon strain was carried out. It was considered necessary to see whether the survival times and clinical symptons obtained in the first experiment could be repeated and also whether the virus could be serially passaged. Two groups of five rabbits were inoculated intradermally by a needle which had been contaminated with lesion material from a rabbit infected with the Brecon strain. Those of the first group were killed 6 days after inoculation (Brecon 6 in Table 2). The second was allowed to proceed normally, i.e. to death or recovery (Brecon N in Table 2). A further group of five rabbits were given 0·05 ml. of the original virus material intradermally and the disease allowed to proceed normally (Brecon 0·05 in Table 2).

A close watch was kept on the rabbits and the timing and nature of the clinical symptoms were recorded. It was apparent from the summarized results (Table 2) that there was no significant difference between those obtained in the first experiment (see comparison of strains) and those obtained by serial passage together with reference back to the original material.

DISCUSSION

Previously there has been a report of two strains of attenuated myxoma virus in Great Britain (Fenner & Marshall, 1957). In 1954 a strain of virus was obtained from Sussex which produced a mean survival time of approximately 22 days and which led to some recoveries (no mortality rate is available). In 1955 a sample of myxoma virus was isolated in the Nottingham area which in the hands of Fenner & Marshall (1957) was shown to be a mixture of two strains. The first was very attenuated having a mean survival time of approximately 121 days and there were recoveries. This had a mortality rate of 23 %. The second strain was fully virulent having a survival time of approximately 12 days and there were no survivors.

From the description of the effects of the Sussex strain (Andrewes *et al.* 1956), the attenuated Nottingham strain and the Australian attenuated KM 13 strain

(Fenner & Marshall 1957), it would appear that the field strains described in this paper are more closely related to the Sussex and KM 13 strains than the Nottingham strain, on the basis of clinical symptoms and mean survival time. The mean survival time of the KM 13 and the Sussex strain are identical (21·5 days in laboratory rabbits), but, whereas the two field strains have in general identical clinical symptoms, there is a marked difference in the mean survival time. The Durham strain infected only 3 out of 5 rabbits and the two unaffected ones were found to be susceptible on reinoculation with a virulent strain. The 3 infected rabbits all recovered. In contrast the five rabbits inoculated at the same time with the Brecon strain all became infected and only one recovered. Of a total of 15 rabbits infected with the Brecon strain all but 2 died, this indicates a mortality rate of 87 %. The mortality rate of the KM 13 strain is 88 % (Fenner & Marshall 1957). The mean survival time with the Brecon strain is 26·3 days (mean of all rabbits inoculated with this strain) whereas the M.S.T. for KM 13 is 21·5. However, the mean survival time for the Glenfield strain, as determined in our hands at the same time, was 15 days while in the hands of Fenner & Marshall (1957) it was 10·2 days. A difference in the room temperature could explain this discrepancy. Certainly, Parker & Thompson (1942) showed that continued high temperature gave infected animals a prolonged survival time. Marshall (1959) demonstrated that the temperature of housing after inoculation had a profound effect on the response of the rabbits inoculated in the standard manner. The mean survival time of the Glenfield strain, as determined during a subsequent experiment at the Infestation Control Laboratory, (carried out by P.J.C.) was found to be 11 days. The difference between this result and that obtained at M.R.E. might be explained by the difference in temperature of the two animal houses. However, it should be pointed out that differences in the strain of rabbits might also influence the survival time (see note at the end).

Approximately 8 years have now elapsed since the initial outbreak of myxomatosis in this country. It is very interesting to note that a strain with a high mortality can still be isolated. It has been suggested by Andrewes, Thomson & Mansi (1959) that myxomatosis need not necessarily follow the same pattern in this country as in Australia. That there are differences it cannot be denied, but it is significant that one of the strains of virus described in this paper is almost identical with the Australian KM 13 strain which is the prototype of the Grade III virulence group of virus strains (Fenner & Marshall, 1957) and this is the group which is predominant in Australia at this time and has been for the last 5 years (Fenner 1962). The isolation of these two attenuated strains is interesting on two counts the first of which has already been outlined, the second concerns the difference noted by Fenner & Marshall (1957) between the Australian and the European strains of virus. It was suggested that the European strains produced a much more nodular disease than their Australian counterparts. That the Brecon and Durham strains produce flat lesions may be very significant in the evolution of the virus in Britain. It was on this supposed difference between Australian and European strains that the proposed grading of virulence for the latter was based.

A survey of virus strains present in this country is being made to determine the virulence and clinical symptoms of the predominant strains, to observe whether there is any change from year to year, and to make a comparison with the Australian strains.

SUMMARY

Two strains of myxoma virus isolated from naturally infected wild rabbits have been described. They showed similar clinical symptoms, the most striking feature being the flat primary lesion. In fifteen rabbits the Brecon strain gave a mean survival time of 26·3 days with a range of 17–47 days, and one recovery. Of 5 rabbits inoculated with the Durham strain three became clinically infected and two remained healthy, the latter were found to be fully susceptible on reinoculation. The three infected rabbits all recovered.

The field strains of virus were compared with known myxoma strains—Glenfield, Cornwall, A & H and KM 13. The first three are fully virulent, while KM 13 is attenuated and characteristic of the field strains now being isolated in Australia. The similarity between the clinical symptoms and mean survival time of KM 13 and the Brecon strain was noted.

It is suggested that the two field strains described in this paper may be the first indication that the evolution of myxoma virus in this country is similar to the evolution of the virus in Australia.

One of us (P.J.C.) is greatly indebted to the Director for allowing this work to be carried out at M.R.E. whilst laboratory accommodation was being prepared at Worplesdon. We are grateful for the help and encouragement of Dr J. C. N. Westwood of M.R.E. To Prof. F. Fenner who gave us the Australian strains of myxoma virus and to Mr J. H. Darbyshire of the Central Veterinary Laboratory who supplied the tissues from which the two attenuated strains were isolated, we extend our thanks.

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EXPLANATION OF PLATE

Lesions in rabbit after inoculation with two strains of myxoma virus.

Fig. 1. (*a*) A & H strain, 15th day, rabbit died at 21 days. (*b*) Brecon strain 15 day, rabbit recovered.

Fig. 2. Brecon strain, 6th day.

ADDITIONAL NOTE BY P. J. C.

Subsequent experiments at the Ministry of Agriculture's Field Research Station gave shorter survival times for all strains tested. In the case of Glenfield the mean survival time (M.S.T.) was 11 days (range 8–13) on one occasion and 11·6 (range 9–14) on another. The Brecon strain gave a M.S.T. of 16 days (range 12–18) and the Durham strain a M.S.T. of 18·4 (range 16–20). The symptoms remained the same In the case of the two field strains the primary lesions were flat and red while the Glenfield strain primary lesion was very raised with a purple necrotic centre. There were no survivors in any of the groups of five rabbits which were used for testing each strain.

The differences in the survival times found at the two establishments are not surprising if the following factors are borne in mind. The animal house at M.R.E. has a fairly high (*c.* 18·5° C. (65° F.)) temperature which is easily maintained through the medium of air conditioning, and double glazing. At the Field Research Station the animal house is a wooden structure, with a certain amount of insulation built in, heated by tubular electric heaters, but with the windows left open to provide ventilation. Therefore, it is much more susceptible to the vagaries of the external environment and in addition the thermostat is set at 15·6° C. (60° F.).

The animals used in the experiments at the field station came from a different source than those used at M.R.E.

That both these factors can influence the survival times has been very well shown by Marshall (1959).

It is interesting to note that the M.S.T. for the Glenfield strain, as determined at the Field Research Station is almost the same as that obtained by Fenner & Marshall (1957)—who quote a figure of 10·2 days (range 9–12).

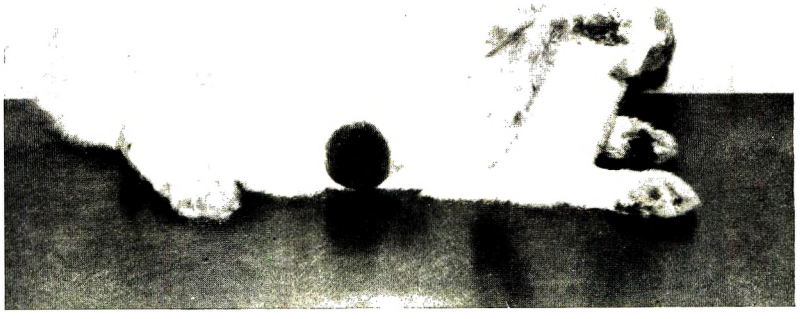

Fig. 1 (*a*)

Fig. 1 (*b*)

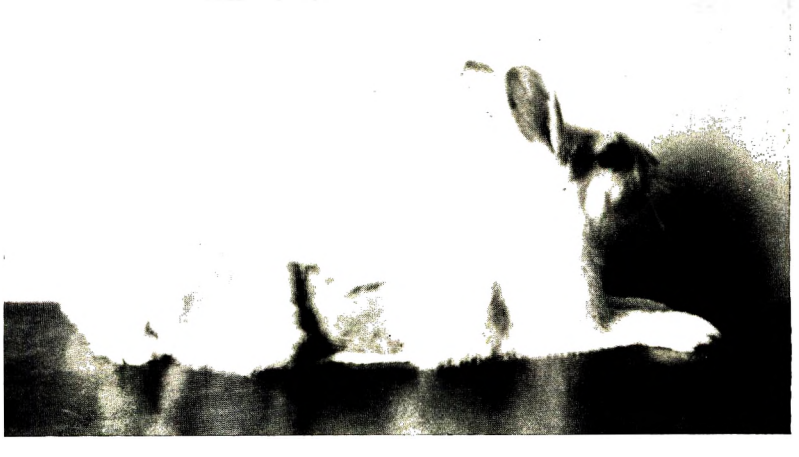

Fig. 2

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Combined use of rodenticidal dust and poison solution against house-mice (*Mus musculus* L.) infesting a food store

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INTRODUCTION

Laboratory and small scale field trials have shown that some measure of control over house-mouse (*Mus musculus* L.) populations living in dry environments could be achieved by surrounding water baits with a dust containing 1% warfarin [3-(α-acetonyl-benzyl-4-hydroxycoumarin)] (Rowe, 1957). The effectiveness of warfarin dust alone in controlling house-mice infesting the wall cavities of food stores was also demonstrated. Both methods have been used recently in an attempt to control a heavy infestation of mice living in the stacks of flour and the wall cavities of a large food store. Bait-boxes containing a water fount surrounded by 1% warfarin dust were placed around the bases of the stacks and in some of the air-lanes dividing stacks. More warfarin dust was laid at the ends of roof trusses and in wall crevices.

Monthly census baitings showed that mice infesting the walls were soon controlled, but that control of the mice living in the stacks was less rapid. It was considered that a quicker kill would have been obtained had the stacks been smaller (200–400 tons) and had the air-lanes been wide enough to allow a bait-box to be placed in each of them. It was also considered that replacing the water in the drinking founts with anticoagulant in solution (Rowe, 1961) might yield quicker results. A second trial against mice infesting a similar store is described below.

METHOD

The store chosen housed approximately 3000 tons of flour built in seventeen stacks measuring between 30 × 30 × 12 ft. and 35 × 30 × 12 ft. During the experiment a turnover of the stock was in progress, some flour stacks being dismantled and replaced by new ones.

An index of the degree of infestation by mice was obtained by test-baiting the stacks, wall ledges and machinery with wheat grains laid on bait trays. Three grains were placed on each tray and the number removed was recorded 24 hr. later; the number of grains was then made up to 3 per tray and the number missing again recorded after a further 24 hr.

Immediately after the first test-baiting was concluded the stacks were treated with poison placed in bait-boxes measuring 11½ in. square (Pl. 1, fig. 1). Each box consisted of a hardboard base with two wooden sides nailed opposite each

other and a detachable hardboard lid. Shallow wooden battens were fixed across the two open ends of the base to prevent dust from spilling. The lid had a 3 in. diameter hole in the centre and rested on the wooden sides. A box was prepared by removing the lid and covering the base to a depth of approximately $\frac{1}{4}$ in. with 1 % warfarin dust. A small chick-fount—consisting of a 1 lb. jam jar and metal base—was partly filled with a solution of sodium warfarin and placed in the centre of the dust. The lid was then put back, the top of the jar projecting through the hole in the lid. The poison, a 0·005 % sodium warfarin/5 % sugar solution, was prepared by adding individual packets of the anticoagulant, contained in a diluent, to water containing 5 % of sugar. The solution was mixed in bulk in a pail and each fount three-quarters filled by immersing the fount-base and jar in the solution.

The flour stacks were supported off the floor on dunnage consisting of a lattice of wooden planks over an open network of bricks. Wherever possible the boxes were partially inserted between the bricks (Pl. 1, fig. 2) with the solid sides placed in line with the length of the stacks. Boxes laid in this manner were found to be less liable to be knocked accidentally than those which projected completely into the gangways.

One box was laid mid-way along each side of the five stacks situated at the ends of the store. Around each of the other twelve stacks an additional box was placed in the 2 ft. air-lane dividing it from its neighbour, the four boxes in the air-lanes being staggered. A further sixteen boxes, making a total of ninety-six, were distributed at eaves height at the gable ends of the store and on the floor in each corner of the building.

During the poison treatment test-baitings were carried out at intervals. At each test-baiting, and periodically between baitings also, the warfarin dust in the boxes was examined; more was added when required and footprints were erased. The chick-founts were also inspected and the poison solution topped up when necessary.

During the early part of the treatment the location of dead mice was recorded daily and during each test-baiting and at each examination of the boxes the store was searched for bodies. The numbers of mice found during the breaking down of stacks were also noted.

RESULTS

The test-baitings

Examination of past baiting records showed that the flour store had had a persistent mouse infestation for nearly 2 years prior to the reported experimental treatment. In the last 16 months of this period between March 1960 and July 1961, baits containing 0·025 % warfarin in medium oatmeal were distributed on the stacks at the rate of one bait per ton of flour. The results of test-baitings with wheat grains at approximately 3-monthly intervals during this treatment are given in the upper part of Table 1.

The average number of wheat grains removed at the test-baiting in July 1961 was 2438 or 44·8 % of the total grain laid. The warfarin/medium oatmeal baits were then removed and the boxes containing 1 % warfarin dust and a 0·005 % sodium warfarin and 5% sugar solution were placed around the stacks. The results of three

further test-baitings done during the dust/liquid poison treatment are given in the lower part of Table 1. After 5 weeks' poison treatment, the number of grains missing was 132 (test-baiting 1) indicating that the mouse population had been reduced by approximately 95 %. Five weeks later the degree of control achieved

Table 1. *Test-baiting results for two different warfarin treatments against mice infesting stacks of flour*

Month of laying test-baiting	Number of wheat grains laid	Number of wheat grains removed (mean of two days)	Percentage removed
March 1960	10,248	3,369	32·9
June 1960	6,515	472	7·2
September 1960	5,070	1,459	28·8
January 1961	5,559	2,643	47·5
April 1961	5,385	2,173	40·3
July 1961	5,445	2,438	44·8
August 1961	5,439	132	2·4
September 1961	5,271	52	1·0
November 1961	5,763	3	0·1

Table 2. *Results of test-baiting*

Location of census points	Number of grains removed daily							
	Pre-treatment test-baiting 4. vii. 61– 5. vii. 61		Test-baiting 1 9. viii. 61– 10. viii. 61		Test-baiting 2 14. ix. 61– 15. ix. 61		Test-baiting 3 22. xi. 61– 23. xi. 61	
Stack A	158	247	62	63	6	14	0	0
B	64	135	0	3	2	1	0	1
C	31	60	0	4	0	4	0	0
D	141	240	1	3	0	0	0	0
E	152	214	2	4	7	22	0	0
F	203	266	0	0	0	0	0	0
G	113	192	0	7	2	0	—	—
H	181	224	0	0	—	—	0	0
I	—	—	—	—	6	0	0	0
J	165	170	0	16	0	0	0	0
K	181	196	0	0	0	0	0	0
L	144	189	0	6	0	0	4	0
M	81	142	14	24	11	16	1	0
N	—	—	—	—	0	2	0	0
O	73	117	—	—	—	—	0	0
P	138	169	20	32	4	4	—	—
Q	170	223	0	0	0	0	0	0
Wall ledges	46	49	3	0	0	0	0	0
Cased goods	0	2	0	0	0	3	0	0
Total	2041	2835	102	162	38	66	5	1
Mean of two days	2438		132		52		3	
Total number laid daily	5445		5439		5271		5763	

had risen to about 98% (test-baiting 2) and after a further 10 weeks (i.e. after the treatment had been in progress for 20 weeks) almost 100% control had been obtained (test-baiting 3). The test-baiting figures for the individual stacks, wall ledges and cased goods are given in Table 2.

Mouse activity at the bait-boxes

Footprints in the warfarin dust were recorded as either light, medium or heavy. They were designated light when only a few prints were visible, medium when prints were scattered over most of the dust and heavy when the dust had been trampled. Table 3 shows the numbers and intensity of visits to the boxes at intervals throughout the treatment.

Table 3. *The number and intensity of visits by mice to the bait-boxes at intervals during the poison treatment*

Date	Interval between inspections (days)	Intensity of footprints			Number of bait-boxes entered (out of 96)
		Light	Medium	Heavy	
5. vii. 61–6. vii. 61	1	11	10	1	22
6. vii. 61–20. vii. 61	14	28	24	29	81
20. vii. 61–21. vii. 61	1	26	10	0	36
21. vii. 61–9. viii. 61	19	32	14	5	51
9. viii. 61–10. viii. 61	1	4	0	0	4
10. viii. 61–13. ix. 61	34	25	1	0	26
13. ix. 61–14. ix. 61	1	1	0	0	1
14. ix. 61–4. x. 61	20	18	2	0	20
4. x. 61–5. x. 61	1	0	0	0	0
5. x. 61–16. x. 61	11	41	11	2	54
16. x. 61–6. xi. 61	21	33	9	0	42
6. xi. 61–21. xi. 61	15	28	5	0	33
21. xi. 61–22. xi. 61	1	0	0	0	0

After 24 hr. 22 of the 96 boxes had been entered by mice. All but one of them had footprints of light or medium intensity. A further inspection 2 weeks later showed that all but eight boxes had by then been entered. Further inspection on 2 consecutive days in each month from late July until mid-September showed that there was a progressive decrease in the number of visits by mice to the boxes. Single inspections in mid-October and early November indicated some renewal of activity, but 80% of the footprints were recorded as light, and no mice entered the boxes at all during 24 hr. from 21 to 22 November—the period of the final test-baiting.

Dead mice recovered

Two dead mice were found 4 days after the beginning of the treatment and 48 during the 1st month; 15 more bodies were found during the remaining 10 weeks of the treatment. Of the 63 dead mice recovered outside the stacks, 48 were found on the floor close to or between stacks, 10 on the tops of stacks, 2 at the base of the inner wall of the store, 2 beneath boxes placed at eaves height, and 1 underneath

machinery. It is probable that many more mice died, for during the dismantling of two of the flour stacks 34 bodies were found. A few recently killed mice found in nests in the centre of one stack showed that at least some of the animals living furthest away from the boxes were visiting them.

DISCUSSION

The test-baiting figures in Table 1 show that, although the poison treatment using cereal/warfarin baits was fairly successful in its early stages, poison baitings between September 1960 and July 1961 proved less effective and the mouse infestation actually began to increase. The greatly improved control obtained when solid warfarin bait was replaced by the bait-boxes containing poison dust and liquid is clear from the lower part of Table 1. The effectiveness of the latter treatment may also be compared with that of the similar one mentioned earlier, where plain water was used instead of the poison solution. The control achieved with the dust and poison solution after 5 weeks' treatment was 95 %—compared with only 85 % after 9 weeks with dust and plain water bait. The supposition that the increased rate of kill in the former treatment was due in part to consumption of the poison solution is corroborated by the fact that patches of dust were found from time to time on the rim of the fount bases.

The experience of both trials showed that the dust/liquid method of poisoning mice requires fairly constant attention and regular maintenance of the baiting points. It was found necessary to top up some of the drinking founts after the treatment had been in progress for only 5 weeks and all were replenished to a varying extent over the next 3 months. After 1 month also, it was noticeable that the poison solution in some of the founts had become slightly cloudy; and after 3 months mould growth was sufficiently advanced to warrant cleansing of the jars and founts. Another difficulty encountered was the need during turn-over to move a number of the boxes from dismantled to new stacks. It was found, however, that with care this could be done with little spilling of the poison solution.

A field trial of the usefulness of the dust/liquid baiting technique against mice living in colder and less dry conditions than encountered hitherto has also been attempted. The turn-over of the stocks caused the trial to be abandoned but the result obtained—a 65 % reduction of the mouse population after 5 weeks' treatment—suggests that the method is less effective under these circumstances. Although an additional bait such as whole wheat placed in the boxes might help to attract the mice more regularly to the baiting points, the dust/liquid baiting method would seem to be most effectively used as an alternative or additional form of control to cereal warfarin baits against mice living in relatively warm environments where alternative drinking supplies are intermittent or non-existent.

SUMMARY

1. A food store infested with house-mice (*Mus musculus* L.) was treated with boxes containing 1 % warfarin dust surrounding a solution containing 0·005 % sodium warfarin and 5 % sugar. The boxes were placed around the bases of the

stacks, in the air-lanes between stacks and at eaves height at the gable ends of the store.

2. Census baitings with counted numbers of wheat grains showed that the mouse population was reduced by about 95 % after 5 weeks of the poison treatment and by almost 100 % after 20 weeks.

3. The field trial suggests that the combined use of rodenticidal dust and poison solution baiting points is a useful additional method of controlling *M. musculus* and is most effectively employed against mice living in warm, dry environments.

Thanks are expressed to our colleague Mr E. W. Powell, who co-operated in this work.

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EXPLANATION OF PLATE

Fig. 1. Bait-box containing 1 % warfarin dust surrounding a 0·005 % sodium warfarin/5 % sugar solution.

Fig. 2. Bait-box with lid removed placed at the base of a flour stack.

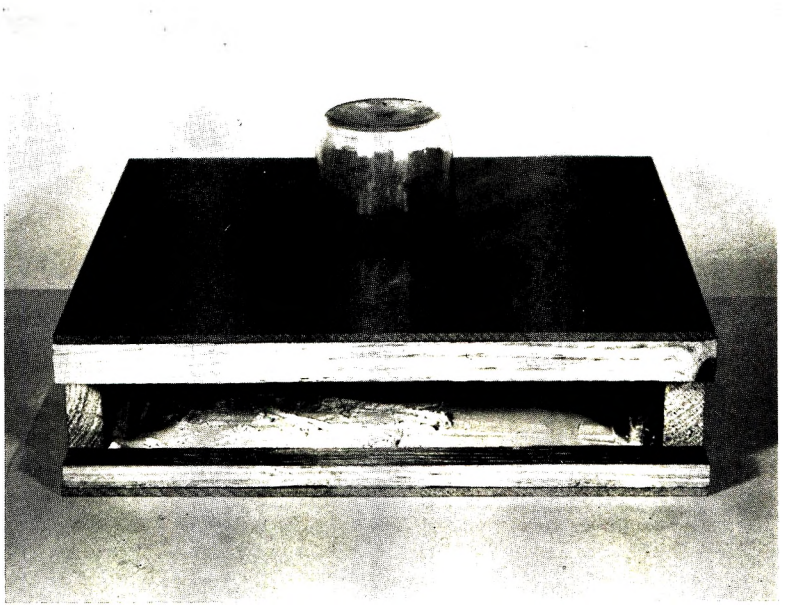

Fig. 1

Fig. 2

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A milk-borne outbreak of food poisoning due to *Salmonella heidelberg*

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INTRODUCTION

An explosive milk-borne outbreak of *Salmonella heidelberg* infection due to unpasteurized tuberculin-tested milk occurred in Cirencester in November 1961 and was traced to a cow with a symptomless salmonella mastitis. The origin of the infection was thought to be contaminated animal feeding stuffs.

Salm. heidelberg infection is rare in cattle and the organism has not been previously reported as a cause of milk-borne salmonellosis. For this reason and because animal feeding stuffs were implicated as the origin of the infection it is considered that the outbreak is of sufficient interest to be recorded.

The veterinary aspects of the outbreak have been discussed by Davies & Venn (1962).

THE OUTBREAK

Between 3 November and 3 December 1961 there were 56 reported incidents* of *Salm. heidelberg* infection, comprising 77 cases and 46 symptomless excreters, in Cirencester and the surrounding rural district. The dates of onset of symptoms of the first or only case in 51 of these are shown in Fig. 1. In three incidents the dates of onset were not recorded and two incidents comprised only symptomless excreters.

During the period of the outbreak 53 other incidents of 'food poisoning' were reported in the area, but salmonellae were not isolated from 131 patients examined. These incidents were unrelated to the *Salm. heidelberg* outbreak and the disease was probably 'epidemic' or non-specific diarrhoea and vomiting which had been prevalent in the district during the late summer.

* Incident means either a single case or a group of 2 or more cases in a household.

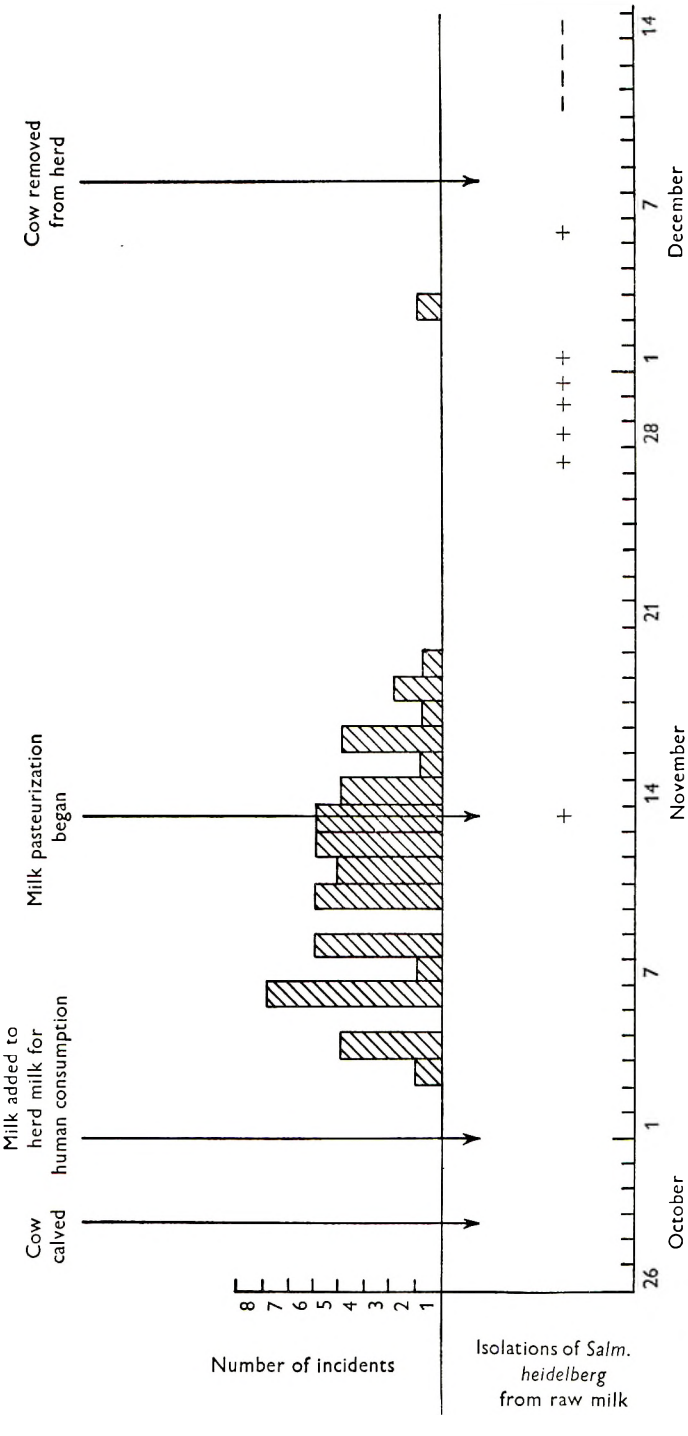

Fig. 1. Dates of onset of symptoms of first or only case in each incident. History of infected cow and isolations of *Salmonella heidelberg* from raw milk. +, *Salm. heidelberg* isolated; —, salmonellae not isolated.

Clinical features

Seventy-two of the 77 patients from whom *Salm. heidelberg* was isolated had diarrhoea and in 36 vomiting was recorded. Abdominal pain was prominent in 16 patients, one of whom, a girl aged 8 years, was admitted to hospital because of suspected appendicitis. A girl aged 7 months had convulsions at the onset of the illness. There were no deaths.

The ages of the 77 cases and 46 symptomless excreters are given in Table 1. Fifty-nine of the 123 cases and excreters were males and 64 females. Thirty-eight of the 77 cases were males and 39 females.

Table 1. *Ages of cases and symptomless excreters*

Age (years)	Numbers of cases and symptomless excreters: Total		Cases		Symptomless excreters	
0– 4	41 (33)		36 (47)		5 (11)	
5– 9	27 (22)		18 (23)		9 (19)	
10–14	19 (16)		8(10)		11 (24)	
15–19	3	36 (29)	2	15 (20)	1	21 (46)
20 and over	32		12		20	
Not known	1		1		0	
Total all ages	123 (100)		77 (100)		46 (100)	

Percentages are given in parentheses.

Milk—the vehicle of infection

The first two notifications of cases in the outbreak were on 10 November; further cases were reported 3 days later. Raw tuberculin-tested milk from a local dairy A had been supplied to all the households concerned. Commencing on the evening of 13 November all milk sold by the dairy was pasteurized and after 19 November only one further incident occurred (see Fig. 1).

Subsequent investigations revealed that in 53 of the 56 incidents milk had been obtained from dairy A. In one incident, a boy aged 2 years whose family obtained milk from a different dairy had consumed milk from dairy A on several occasions when he had visited his grandmother. In the remaining two incidents there was no apparent association with milk from dairy A.

The dairy

Dairy A was supplied daily from four farms with about 300 gal. of milk, which was bottled and distributed to 1260 families in and around Cirencester. *Salm. heidelberg* was isolated from milk sampled on 13 November from three churns from farm B but not from samples from 17 churns from the three other farms.

Farm B supplied daily about 80 gal. of Guernsey milk, which on any one day was distributed to about 350 families. The milk was probably contaminated on several occasions between 1 and 13 November and in this period it was sold to about

600 families. The number of families at risk may have been greater than 600 because occasionally there was more Guernsey milk than was required and the excess milk from farm B was added to the milk from the other farms. In addition cross-contamination of the milk from the other farms might have occurred in the bottling machine. Fifty-eight families were known to have been affected in the outbreak but others may not have been reported. Taking into account these factors it is estimated that about 10 % of the families at risk were affected.

There were six people employed at dairy A, one of whom developed diarrhoea on 6 November and two of whom were found to be symptomless excreters of *Salm. heidelberg*. There were three other cases and seven symptomless excreters in their families. These cases occurred in the outbreak and the excreters were probably infected at the same time.

The farm

The source of infection on farm B was found to be a cow with mastitis. *Salm. heidelberg* was isolated from the udder post mortem (Davies & Venn, 1962). This cow was born on the farm in February 1956 and had never been ill.

The cows were milked by machine and the milk was poured into churns through a strainer containing a cellulose filter pad. *Salm. heidelberg* was isolated from milk from 9 of 20 churns sampled at the farm and from two cellulose filter pads after they had been used. The churn containing milk from the infected cow and the churn next filled were found to be contaminated on four occasions, but when a new filter pad was used for each churn only the first churn was positive. It seems therefore that spread of the organism to the second and possibly to subsequent churns was due to contamination of the strainer and filter pad. The infected cow was slaughtered on 8 December and *Salm. heidelberg* was not subsequently isolated from 64 milk samples collected over a period of three months.

Davies & Venn (1962) have discussed how *Salm. heidelberg* may have reached the udder of the cow, either by infection through the teat canal or by ingestion and septicaemic spread from the intestine. How the organism reached the cow remains to be considered.

It seems unlikely that the infection was brought into the farm by a carrier animal because the only recent addition to the cattle herd was a bull purchased in 1959, and the herd had had no contact with other animals. Salmonellae were not isolated from faecal specimens of cows, sheep and chickens on the farm. Pigs were not kept and there was no possibility of contact with pigs on neighbouring farms. The only other animal infected was a calf, born after the outbreak, which was fed on the contaminated milk (Davies & Venn, 1962).

There was no evidence that any of the four farm workers or their families were excreting the organism before the outbreak. One child in each family developed symptoms during the outbreak and subsequently three of the workers and three other family members were found to be infected. These ten persons had all consumed the contaminated milk, whereas two of four family members not infected did not consume the milk. The cases and symptomless excreters all ceased to excrete the organism within 3 months of the outbreak and it seems

reasonable to conclude that none of them was a chronic carrier and that they were all infected in the outbreak at the same time.

Before August 1961, the cattle were daily driven past a cesspit which drained the farm workers' cottages, and as this occasionally overflowed the cows may have had access to the sewage. It is unlikely that the sewage was contaminated with *Salm. heidelberg* before the outbreak, because none of the occupants of the cottages could be shown to be a chronic carrier; moreover, it is improbable that anyone in the cottages was infected twice, once before the outbreak and again in the outbreak.

In 1961 there were 289 reported incidents of *Salm. heidelberg* infection in England and Wales (Report, 1962), the nearest case being in Bristol about 30 miles from Cirencester. None of the farm workers or their families could recall having had symptoms before the outbreak which could be attributed to infection with *Salm. heidelberg* nor had they visited Bristol or other districts at the time the reported cases occurred. Pork and pork products have been suspected as the source of several outbreaks in the past but the farm workers and their families were not in the habit of eating such meat nor had they eaten foods processed by producers previously implicated in any outbreaks.

A sewer from a neighbouring hutted camp for Polish refugees traversed the farm but it had not been blocked nor was there evidence of leakage of sewage and it could not therefore have been the origin of the infection.

The cattle herd was supplied with chlorinated water direct from the mains and had no access to ponds or streams. A sample of mains water from the cowshed on 12 December contained no salmonellae and 12 routine samples collected during the year from the source of supply to the area had presumptive coliform counts of nil per 100 ml.

Animal feeding stuffs

Animal feeding stuffs manufactured by the firm which supplied cattle cake to farm B were found to be contaminated with several salmonella serotypes; *Salm. heidelberg* was isolated from English meat and bone meal at the supplier's factory. This serotype is uncommon in animal feeding stuffs (Walker, 1957; Reports, 1959*a*, 1961) and the isolation of the organism from the cow and from material at the factory suggested that the two might be associated.

The dairy cows were fed on cattle cake which contained vegetable and inorganic mineral ingredients—it did not contain animal protein. Two of 23 samples of cattle cake and one of 62 samples of various vegetable ingredients, sampled at the factory, were contaminated with salmonellae (Table 2). These samples were obtained from batches made after the outbreak. Twenty-one samples of inorganic minerals and 26 samples of calf and poultry foods were negative. Salmonellae were isolated also from sacking and sack dust from bags which had contained cattle cake.

At the factory where the cattle cake was made, English meat and bone meal and a protein concentrate were used as ingredients of other feeding stuffs. Salmonellae were isolated from both these materials—*Salm. heidelberg* from

English meat and bone meal. The same machine was sometimes used to mix these materials as was used to mix the ingredients of the cattle cake, so that cross-contamination could easily have occurred in the machine.

Table 2. *Isolations of salmonellae from animal feeding stuffs, sacking and sack dust November* 1961–*May* 1962

Type of material	Number of samples examined	Number of samples positive	Salmonella serotypes
Cattle cake	23	2	cubana, kiambu
Calf and poultry foods	26	0	—
Cattle cake ingredients			
(1) Vegetable materials	62	1	taksony
(2) Inorganic minerals	21	0	—
Sacking and sack dust	24	2	cubana, taksony
Protein concentrate	18	2	oranienburg, seftenberg
English meat and bone meal	15	1	heidelberg
Total all materials	189	8	

The materials were sampled at an animal feeding-stuffs mill and a factory where used sacks were cleaned.

The English meat and bone meal was made in Bristol from butchers' scraps, offal, and meat and bone from abbattoirs and knackers' yards in the west of England.

A sample of knacker meat obtained from a pet food shop in Bristol in August 1961 was found to be contaminated with *Salm. heidelberg*. This suggests that animal infection was present in the area and, although it was not possible to determine the farm of origin of the infected meat, the raw materials of the meat and bone meal might well have been contaminated with *Salm. heidelberg* before the Cirencester outbreak.

It is not clear how the animals from which the meat and bone meal was made came to be infected in the first place, but the organism may have been introduced in some material such as an imported animal feeding stuff. The cycle of: infected animals—contaminated meat and animal products—contaminated animal feeding stuff—infected animals, would then have led to the perpetuation of the infection in the area.

LABORATORY METHODS

Faeces specimens from patients were cultured on deoxycholate-citrate-agar (D.C.) plates (Hynes, 1942) and in selenite F broth (Hobbs & Allison, 1945), and non-lactose-fermenting organisms obtained from these media were tested biochemically. Organisms giving the reactions of the salmonella group were then typed by serological methods.

Samples of milk were examined by an enrichment method. Fifty ml. of milk was added to an equal volume of double-strength Leifson's selenite F medium and incubated for 18 hr. The culture was then plated on deoxycholate-citrate-agar and non-lactose-fermenting colonies were picked off for biochemical and serological

tests. Water samples were examined by the same enrichment method and presumptive coliform counts were also performed.

Animal feeding stuffs were collected from previously unopened containers using sterile spoons and the specimens forwarded to the laboratory in polythene bags. Twenty g. samples—four from each bag—were added to 100 ml. amounts of Ringer's solution and incubated at 37° C. for 2 hr. To each of these 100 ml. of double-strength selenite F broth was then added and incubation continued overnight. Subcultures were made on to D.C. plates and incubated for 24 hr., after which non-lactose-fermenting colonies were picked off and examined serologically and biochemically.

DISCUSSION

Milk-borne outbreaks of salmonellosis are not common in this country (Taylor, 1960), but nevertheless over the past 20 years the number of known cases in the recorded outbreaks was nearly 3000 (Table 3) and the actual number of cases was certainly much greater. Most of the outbreaks were due to contaminated raw milk from sick or carrier cows and could have been prevented by pasteurization. The outbreak described in this paper emphasizes again the need for the pasteurization of all milk.

It is considered that the origin of this outbreak was contaminated animal feeding stuffs. Wright, Norval & Orr (1957) suggested also this source of infection in a milk-borne outbreak of *Salm. thompson* in Edinburgh in 1956. In an outbreak of *Salm. dublin* in Somerset in 1952, surface water contaminated by cattle on a neighbouring farm was a possible source (McCall, 1953). In no other reported outbreak of salmonellosis was there evidence to suggest how the cattle became infected.

It is of interest to consider the salmonella serotypes isolated in milk-borne outbreaks in the United Kingdom in the past 20 years (Table 3). *Salm. dublin* was the most common organism until 1950, after which it was replaced by *Salm. typhimurium*. More recently other less common serotypes have appeared.

The most likely origin of *Salm. dublin* is the cattle themselves because the organism is primarily a bovine pathogen and is uncommon in other species and in animal feeding stuffs (Taylor, 1960). However, *Salm. typhimurium* and the other serotypes are much less host specific and are common in other species and in animal feeding stuffs (Walker, 1957; Reports 1959*a*, 1961); cattle are therefore less likely to be the origin of the infection. It is possible that the change in the salmonella serotypes causing milk-borne outbreaks may be related to the increased use of imported animal feeding stuffs in this country in recent years.

There has also been a change in the seasonal incidence of the reported milk-borne outbreaks (Table 3). Most have been between April and September but, since 1953, six outbreaks have occurred in the colder weather between October and February—three of them in January and February. The reason for this is not apparent but it might be related to seasonal variations in the use of animal feeding stuffs.

Table 3. *Reported milk-borne outbreaks of salmonellosis in the United Kingdom*, 1942–61

Date	Salmonella serotype	Number of persons infected	*Type of milk	Probable source of infection	Authors
Aug. 1943	dublin	162	Raw	Symptomless cow excreting organism in faeces	Sutherland & Berger (1944)
Sept. 1947	dublin	97	Raw	Sick cow which died	Henderson *et al.* (1948)
Sept. 1947	dublin	2 large outbreaks	—	—	Report (1948)
May 1949	dublin	50	Raw	Sick cow which died	Cromb & Murdock (1949)
May 1949	newport	165	Raw T.T.	Sick cow with enteritis	Unpublished P.H.L.S. records
July 1949	typhimurium	337	Raw T.T.	Possibly sick farm worker	Report (1949)
July 1950	dublin	—	Raw	—	Report (1951)
July 1950	typhimurium	—	Raw	—	Report (1951)
July 1951	dublin	2	Fresh	—	Report (1954)
July 1951	typhimurium	2	Fresh	Evidence that milk was responsible not complete	Report (1954)
July 1951	oranienburg	2	Fresh	Evidence that milk was responsible not complete	Report (1954)
Sept. 1952	dublin	610	Raw T.T.	Sick cow with mastitis, possibly infected by contaminated water	McCall (1953)
Feb. 1953	typhimurium	121	Raw T.T.	Symptomless farm worker or cattle	Lennox *et al.* (1954)
June 1953	typhimurium	252	Raw T.T.	Symptomless cow excreting organism in faeces and probably also in milk	Norton & Armstrong (1954)
June 1953	typhimurium	211	Raw T.T. and pasteurized	Sick cattle or farm worker. The pasteurized milk was contaminated by raw milk in the bottling machine	Riddell (1954)
Aug. 1953	typhimurium	13	Raw	Cow	Report (1955)
Aug. 1955	typhimurium	17	Pasteurized	Contaminated after pasteurization by bottle tops soiled with mouse faeces	Report (1956)

Table 3 (*cont.*)

Date	Salmonella serotype	Number of persons infected	*Type of milk	Probable source of infection	Authors
Jan. 1956	typhimurium	6	Raw T.T.	Cow excreting organism in faeces	Report (1957)
Aug. 1956	thompson	11	Raw	Sick cow excreting organism in milk and faeces	Wright *et al.* (1957)
1950–1957	typhimurium	3 outbreaks	—	—	Chalmers & Sampson (1958)
1950–1957	dublin	—	Raw T.T.	—	
1950–1957	enteritidis	320	Pasteurized	Milk contaminated at centre where it was consumed	
June 1957	newport	21	Raw	Sick cow excreting organism in milk	Boyd (1958)
Jan 1958	typhimurium	125	Raw	Sick cattle	Riddell *et al.* (1959)
Aug. 1958	typhimurium	44	Raw T.T.	Symptomless dairy worker	Report (1959*b*)
Sept. 1958	typhimurium	20	Raw T.T.	Sick cow with enteritis	
Sept. 1959	typhimurium	26	Raw	—	Report (1960)
Oct. 1959	typhimurium	3	Raw	Sick calves. Milk probably contaminated by dairy workers who handled the calves	
Oct. 1960	typhimurium	58	Raw T.T.	Sick cow	Whitehead (1961)
May 1961	typhimurium	60	Raw	Cow excreting organism in milk	Report (1962)
July 1961	typhimurium	21	Raw T.T.	Cow with enteritis	Parry (1962)
Sept. 1961	enteritidis var. jena	Several families	Raw	Sick cow	Report (1962)
Nov. 1961	heidelberg	123	Raw T.T.	Symptomless cow excreting organism in milk	This paper

* Outbreaks due to canned or dried milk are not included.

SUMMARY

A milk-borne outbreak of *Salm. heidelberg* infection due to unpasteurized milk from tuberculin-tested cows is described. There were 77 cases and 46 symptomless excreters of the organism. There were no deaths. The infection was traced to a cow with a symptomless salmonella mastitis and the origin of the organism was thought to be contaminated animal feeding stuffs.

We are indebted to Dr Joan Taylor, Colindale, for the identification of the salmonella serotypes; to Dr H. R. Cayton, Bristol, for assistance in the examination of animal feeding stuffs; to Dr E. N. Davey, Gloucester, for permission to include his results of milk examinations; and to the following public health inspectors who assisted in the field investigation—Mr A. Furniss, Mr G. Baillie and Mr M. V. Smith, Cirencester Urban District; Mr H. Wheeler and Mr R. Hayward, Cirencester Rural District; Mr P. Hayter, Bristol; and Mr D. S. Smith, Trowbridge.

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The effect of some antiseptic reagents on the bacterial flora of smallpox lymph

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INTRODUCTION

In previous work (Cardone, 1953, 1956), data were published describing the development of a dehydrated smallpox vaccine made in calf globulin that was more durable than glycerinated vaccines when stored with or without refrigeration. The data included experimental evidence demonstrating that when certain concentrations of phenol and ether were added to lymph vaccine preparations which were ordinarily highly contaminated, the bacterial flora was rapidly diminished (Cardone, 1953).

The lymph used for these experiments, however, was from calves whose inoculated site had been sprayed twice daily with varying concentrations of Roccal* during the viral incubation period. Mention was made that the effects of this treatment might be a bactericidal additive to the action of the phenol and ether which were usually added to the vaccine preparations.

The data that follow derive from further investigations of this factor.

MATERIALS AND METHODS

Materials

Vaccine preparations

These were 20 % lymph emulsions ground in calf globulin as described before (Cardone, 1953).

(*a*) Ten preparations, each made to contain 0·5 % phenol and 0·8 % ether and prepared with a lymph pool derived from calves treated with Roccal during viral incubation period.

(*b*) Ten preparations, each made to contain 0·5 % phenol and 0·8 % ether and prepared with a lymph pool derived from calves not Roccal-treated during viral incubation period.

(*c*) Ten preparations, each made to contain 0·2 % Roccal, 0·5 % phenol and 0·8 % ether and prepared with a lymph pool derived from calves not Roccal-treated during viral incubation period.

* Alkyl dimethylbenzyl ammonium chloride, marketed by The Winthrop Chemical Co., Windsor, Ont., Canada.

Non-viral 'vaccines' for toxicity tests

The skin of normal rabbits was triturated to a fine pulp to simulate smallpox calf lymph. Three vaccines A, B, and C, were prepared with it as usual (Cardone 1953) and each contained antiseptic reagents as follows:

A vaccine, 0·2 % Roccal, 0·5 % phenol, 0·8 % ether;
B vaccine, 0·2 % Cresol Compound N.F., 0·5 % phenol, 0·8 % ether;
C vaccine, 0·5 % phenol, 0·8 % ether.

Methods

Lyophilization

All the vaccines produced, including the non-viral 'vaccines' for toxicity tests, were lyophilized in small ampoules and sealed in vacuo the morning after preparation as described before (Cardone, 1953).

Bacterial counts

Immediately following lyophilization of the smallpox vaccines described under Materials, (*a*)–(*c*), one ampoule from each lot was opened, reconstituted to original volume with sterile distilled water and then diluted 1/25. One ml. of the dilution was mixed thoroughly with 15 ml. of sterile beef heart agar at 43° C., poured in a sterile Petri dish and allowed to solidify. After incubation for 48 hr. at 37° C., followed by 48 hr. at 20° C., the number of colonies on the plate was counted and multiplied by the dilution factor. The resulting figure represented the number of viable organisms per ml. present in the vaccine.

Toxicity tests: rabbit

The non-viral 'vaccines' described under Materials were reconstituted to original volume with sterile distilled water and tested for toxicity as follows:

(*a*) *Rabbit test, patch method.* 1 ml. each of 'vaccines' A, B and C was dispensed to a fourfold, 1 in. square of sterile gauze. The three patches were cellophane-taped in intimate contact with the depilated skin of a rabbit and removed after 6 hr. The test sites were examined for irritation effect after removal of the patches (6 hr.) and again after 24 , 48 and 72 hr.

(*b*) *Rabbit test, scratch method.* 0·2 ml. separately of A, B and C 'vaccines' was scratched into the depilated skin of a rabbit each in an area 2·5 cm. square. The volume of emulsion was spread over the prescribed site and parallel scratches, about 1 mm. apart, were crisscrossed over the area with a sterile needle. Examinations for irritation effect were done as described above.

Toxicity test: human

Six lots of lyophilized smallpox vaccine, prepared as previously described and containing 0·2 % Roccal, 0·5 % phenol and 0·8 % ether, were pooled and reconstituted to original volume with 50 % glycerine solution. Dispensed in one-dose capillary tubes, they were tested for acceptability of viral potency and bacterial count and were sent to clinics to be used for human vaccinations like any other regularly prepared preparation sent for routine testing.

RESULTS AND DISCUSSION

Data of Table 1, upper half, indicate that smallpox vaccines produced with lymph from Roccal-treated animals and containing 0·5 % phenol and 0·8 % ether show rapid diminution of the bacterial flora. The bactericidal activity occurs, usually, within 24 hr. of vaccine preparation and the bacterial flora reduction is such as to be acceptable to the requirements of the National Institutes of Health

Table 1

Adequate bacterial flora diminution occurs when 0·5 % phenol and 0·8 % ether are added to vaccines prepared with highly contaminated lymph from 'Roccal-treated' calves: the reverse is true when similar vaccines are prepared with lymph from calves not treated with 'Roccal'.

Prep. no.	Bacterial count/ml. of lymph used	Bacterial count/ml. of resulting vaccine preparations containing 0·5 % phenol and 0·8 % ether
	Lymph derived from calves treated with 'Roccal' during viral incubation period	
13	200,000	260
18	160,000	300
19	180,000	320
20	250,000	225
22	155,000	210
25	180,000	275
29	190,000	325
32	175,000	150
34	175,000	160
36	195,000	210
	Lymph derived from calves *not* treated with 'Roccal'	
26		
27		
35		
42		
47		
48	Uncountable	Uncountable
49		
107		
108		
109		

(U.S. Department of Health, 1951) and as was reported in previous publications (Cardone, 1953, 1956). Data of Table 1, lower half, on the contrary, show that when the same concentrations of phenol and ether are added to vaccines prepared with lymph from animals not Roccal-treated during the viral incubation period the bacterial flora diminution is not adequate.

These results suggest that lymph from Roccal-treated animals might contain adsorbed quantities of the reagent (Cardone & Mazzarella, 1938) which act as an additive to the bactericidal activity of the phenol and ether.

Table 2

Successful diminution of bacterial flora is obtained when 0·2 % 'Roccal', 0·5 % phenol and 0·8 % ether are added to vaccines prepared with lymph from calves not treated with 'Roccal'.

Prep. no.	Bacterial count/ml. of lymph used	Bacterial count/ml. of vaccine preparations containing 0·2 % 'Roccal', 0·5 % phenol and 0·8 % ether
105	Uncountable	310
110		436
111		140
112		264
113		180
114		120
115		210
116		190
118		290
120		75

Proof of this premise might have been determined readily by direct chemical assay of Roccal in lymph; however, it was found impossible to do so since protein digestion procedures necessary for such work destroy the reagent. A bacteriological approach, then, had to be adopted. It was done by adding to each of a series of vaccines prepared with lymph from animals not Roccal-treated increasing concentrations of Roccal together with the constant concentrations of 0·5 % phenol and 0·8 % ether. Bacterial counts revealed that the vaccine containing 0·2 % Roccal showed a diminution of bacterial flora comparable to that obtained when 0·5 % phenol and 0·8 % ether are added to vaccines prepared with lymph from Roccal-treated animals.

To substantiate this finding some one hundred vaccines were prepared with lymph from animals not Roccal-treated and containing 0·2 % Roccal, 0·5 % phenol and 0·8 % ether. Data of Table 2 not only show the adequate bacterial flora diminution in ten preparations representative of the series, but give evidence that lymph from Roccal-treated animals contains adsorbed, or has the additive bactericidal effects of the use of, at least 0·2 % Roccal when in final vaccine dilution of 20 % emulsion.

The Roccal effect, it has been observed, is variable from animal to animal due to variations in scarification techniques, skin conditions, etc., therefore it has been found preferable to add the recommended concentrations of Roccal, phenol and ether to vaccines at the time of their preparation with lymph from animals not Roccal-treated.

Roccal has been reported to cause no dermatitis in man (Walter, 1938). The

possibility that the combination of Roccal, phenol and ether might cause dermal toxic effects was, however, investigated. Data of Table 3 show that vaccine preparations containing the combination of reagents cause negligible irritation effect in the rabbit and none in sixty-three vaccinated humans.

Table 3

Non-viral 'vaccines' containing 0·2 % 'Roccal', 0·5 % phenol and 0·8 % ether reconstituted from the dehydrated state cause negligible irritation to the skin of the rabbit. In human vaccinations, smallpox vaccines containing the same reagents and reconstituted from the dehydrated state cause no reaction unusual with 'takes'.

	Number of hours from test beginning and degree of irritation at test sites*.			
Type of test	6	24	48	72
I. Rabbit test				
1. Patch method				
A. 'vaccine' containing: 0·2 % 'Roccal', 0·5 % phenol, 0·8 % ether	0	0	±	0
B. (Control) 'vaccine' containing: 0·2 % cresol compound N.F., 0·5 % phenol, 0·8 % ether	0	0	±	0
C. (Control) 'vaccine' containing: 0·5 % phenol, 0·8 % ether	0	0	0	0
2. Scratch Method				
A. 'vaccine', above	0	±	0	0
B. 'vaccine', above	0	±	0	0
C. 'vaccine', above	0	0	0	0

* 0, No reaction; ±, slight reddening of the skin.

II. Human test	Reactions and number of days after vaccination		
	2	7	14
Sixty-three children were vaccinated with a reconstituted lyophilized vaccine pool containing the reagents as in A above	No reactions	No reactions unusual with smallpox 'takes'	

The use of Roccal in the concentration described did not affect the viral potency of the vaccines when tested in the rabbit over a period well over 2 years (Cardone, 1953).

SUMMARY

Data are presented which amplify previously published work on the additive antiseptic effect of Roccal as follows:

1. Smallpox calf pulp derived from animals whose skin is treated with Roccal during the viral incubation period apparently adsorbs sufficient concentration of the reagent to produce a measurable bactericidal effect.

2. The adsorbed antiseptic induces a significant additive effect well beyond

that of the action of stated concentrations of phenol and ether alone, as evidenced by the fact that this combination produces rapid diminution of the bacterial flora in highly contaminated calf lymph.

3. Vaccines containing the combination of antiseptics described caused no dermatitis or other untoward reactions in rabbits and humans and their viral potency was not affected.

4. The use of the antiseptic reagents makes possible the rapid preparation of smallpox vaccines for human use. Acceptable bacterial flora levels are obtained usually within 24 hr. of preparation.

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Adverse reactions in cattle after vaccination with lapinized rinderpest virus

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INTRODUCTION

The endemicity of rinderpest in Kenya was broken by 1947 through the widespread and compulsory use of attenuated goat virus vaccine in native areas during the preceding seven years (Daubney, 1949*a*). Vaccinations are still maintained because of the threat of the reintroduction of the disease by trade cattle and wild game from Ethiopia and Somalia.

In cattle attenuated goat virus induces a well-defined thermal and clinical response which is mild in indigenous stock but can be severe in exotic animals. Consequently considerable interest was aroused by the paper on the more attenuated lapinized rinderpest virus presented by Cheng and Fischman to the 1948 Rinderpest Conference in Nairobi. Ampoules of the Nakamura III strain of lapinized rinderpest virus were obtained, from which Brotherston (1951*a*, *b*) developed the freeze-dried lapinized rinderpest virus vaccine now used in East Africa. The first batches of vaccine were issued in 1949 and its popularity in Kenya can best be judged by the steady increase in annual issues. Only the Sanga type of cattle of Ankole exhibited a clinical reaction after vaccination. Other breeds in East Africa, including purebred European cattle, were successfully immunized without incident (Brotherston, 1951*b*). The vaccine was regarded by field officers as being 'safe'. However, in 1954, 47 previously unvaccinated young Guernsey cattle in one herd developed alarming post-vaccinal reactions. The incident was reported by Brown, Scott & Brotherston (1955), who suggested that the important factor was the low innate resistance of the cattle and not the virulence of the virus. Thereafter an attempt was made to trace the effect in cattle of every dose of lapinized rinderpest virus used in Kenya during the next 30 months. The findings are herein recorded.

MATERIALS AND METHODS

Survey

The expected response of Kenya cattle given lapinized rinderpest virus is an inapparent infection. A reactor was therefore defined as any animal which deviated from normal health following vaccination. Field officers of the Department of Veterinary Services were contacted and asked to supply the following data: the numbers and types of cattle vaccinated, the batch number of the vaccine used,

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the numbers and types of cattle which reacted after vaccination, the rinderpest immunity status of the reacting cattle before vaccination, the ages of the reacting cattle, the blood relationships of reacting cattle within a herd, the lengths of the incubation periods, the lengths of the clinical periods, descriptions of the clinical signs, and the presence or absence of complicating factors such as other vaccinations or intercurrent diseases. In addition, field officers were instructed that all reports of reactions were to be investigated immediately. If the reactions simulated rinderpest the field officer was requested to contact the Veterinary Laboratory, Kabete, either by telephone or telegram. The author visited the farm if there was any hope of isolating virus.

Retests of vaccine batches

Whenever adverse reactions were reported the production protocols of the incriminated batch of vaccine were examined. Sample ampoules of each batch are always held in the laboratory and in the earlier months of the investigation these ampoules were retested in rabbits and cattle.

Virus isolations

Experience revealed that recovery of virus from suspect blood samples was only successful if the samples were fresh and in later investigations rabbits were taken to the farm and inoculated intravenously on the spot. The tissues of rabbits which reacted were harvested and emulsified. Part of the emulsion was freeze-dried and stored and part was inoculated subcutaneously into Kenya grade cattle whose subsequent response was carefully recorded. Inoculated cattle were housed with an equal number of susceptible cattle.

RESULTS

Incidence of adverse reactions

Between 1 January 1954 and 30 June 1956, 310,971 cattle were vaccinated with lapinized rinderpest vaccine in Kenya. Post-vaccinal reactions occurred in 278 cattle, giving an incidence rate of 0·09 %. Twenty deaths attributable to vaccinations occurred, giving a post-vaccinal mortality rate of 0·006 % and a case mortality rate of 7·2 %. Four types of reactions were observed. In descending order of incidence they were 'rinderpest-like' reactions, aggravations of latent protozoal infections, transient spermatolysis and anaphylaxis (Table 1). In addition, blackquarter occurred in 9 cattle on two adjacent farms after vaccination. A case of heartwater which terminated fatally 7 days after vaccination may have been an aggravation of a latent infection or a chance simultaneous development of acute heartwater.

Genuine rinderpest-like reactions affected 217 cattle, giving an incidence rate of 0·07 %. The mean incubation period was 6·9 days with 95 % fiducial limits of $\pm$ 1·7 days. The clinical signs were characteristic of rinderpest and varied from transient thermal reactions together with slight lachrymation and slight diarrhoea to severe reactions with temperatures up to 107° F., marked foetid diarrhoea and

erosions of the oral mucosa. The most common sequel was a marked loss in condition which lasted for several weeks. The next commonest sequel was abortion. One hundred and twenty-one of the 217 reactors were pregnant and 72 aborted, giving a case abortion rate of 60 %. However, 61 of the abortions occurred in one herd, which was later shown to be riddled with venereal and genital diseases. Elsewhere the case abortion rate was 18 %. Deaths were rare and only one genuine rinderpest-like death was observed, giving a case mortality rate of 0·5 %. The over-all post-vaccinal mortality rate associated with rinderpest-like reactions was 0·0003 %. A characteristic of the incidence of rinderpest-like reactions was the tendency for several animals to be affected in the same herd. The mean percentage of the number of cattle which reacted within reacting herds was $17{\cdot}0 \pm 8{\cdot}1$.

Table 1. *Incidence of adverse reactions in Kenya cattle after vaccination with lapinized rinderpest virus*

Year	Cattle vaccinated	Rinderpest-like reactions	Aggravations of protozoal infections	Transient spermatolysis	Anaphylaxis	Total
1954	110,645	99	16	—	1	116
1955	100,612	61	8	6	—	75
1956	99,714	57	28	—	2	87
Total	310,971	217	52	6	3	278

Aggravations of latent diseases were observed in 52 animals. The case distribution was sporadic. Nineteen deaths occurred, giving a case mortality rate of 36·5 %. Protozoal infections implicated were anaplasmosis, babesiasis and theileriasis.

Transient spermatolysis developed in six vaccinated Red Poll bulls on one farm. The standard of bull management was excellent and daily records were kept of each bull's temperature. After vaccination none of the bulls exhibited a thermal or clinical reaction. However, semen samples taken on the 5th through to the 14th day after vaccination were abnormal and the spermatozoa were non-motile. Thereafter the motility returned.

Anaphylactic shock occurred immediately after vaccination in three animals. Two were Sahiwal-zebu crossbreds. The third was a low grade Aberdeen-Angus steer.

Incidence within species

Data are recorded in Table 2. The vaccinated cattle were *Bos indicus* and *Bos taurus* and the majority were crosses between the species. The incidence of rinderpest-like reactions was highest in high grade *Bos taurus–Bos indicus* crosses (Table 3). The difference between the incidence rates in high-grade *Bos taurus–Bos indicus* crosses and *Bos taurus* was not however significant ($\chi^2 = 1178$, $P > 0{\cdot}20$) whereas the difference in incidence between high and low grade cattle was significant ($\chi^2 = 75{\cdot}504$, $P < 0{\cdot}001$). Rinderpest-like reactions did not occur in 87,787 vaccinated *Bos indicus*.

Aggravations of latent protozoal infections were not observed in *Bos indicus*.

The incidence of aggravated latent infections was highest in *Bos taurus* (Table 3). The incidence rates in high and low grade *Bos taurus–Bos indicus* crosses were similar ($\chi^2 = 0{\cdot}316$, $P > 0{\cdot}50$) and were significantly lower than in *Bos taurus* ($\chi^2 = 61{\cdot}757$, $P < 0{\cdot}001$). A re-investigation revealed that only pedigree imported *Bos taurus* cattle were involved and that they had all been inoculated with *Babesia* and *Anaplasma* prior to rinderpest vaccination. None of the pedigree cattle died. Fifteen (58 %) of the high grade *Bos taurus–Bos indicus* reactors died. Four (29 %) of the low grade *Bos taurus–Bos indicus* reactors died.

The transient spermatolysis was noted in *Bos taurus* bulls. The anaphylactic reactions occurred in two *Bos indicus* and in a low grade *Bos taurus–Bos indicus* cross.

Table 2. *Species incidence of adverse reactions in cattle after vaccination with lapinized rinderpest virus*

Species	Number of cattle vaccinated	Number of reactors: Rinderpest-like reactions	Aggravations of latent disease	Transient spermatolysis	Anaphylaxis
Bos indicus	87,787	0	0	0	2
Bos taurus	4,043	6	12	6	0
Bos taurus × *Bos indicus*					
High grade	75,784	188	26	0	0
Low grade	51,828	23	14	0	1
Unclassified	91,529	0	0	0	0

Table 3. *Incidence rates of adverse reactions in different species of cattle vaccinated with lapinized rinderpest virus*

Species	Percentage incidence: Rinderpest-like reactions	Aggravations of latent disease
Bos indicus	0	0
Bos taurus	0·15	0·30
Bos taurus × *Bos indicus*		
High grade	0·25	0·03
Low grade	0·04	0·03

Incidence within breeds

Data are recorded in Table 4. Insufficient numbers of cattle of the Galloway, South Devon, Sussex and Swiss Brown breeds were vaccinated for valid conclusions to be drawn regarding the true incidence of post-vaccinal reactions in these breeds.

Rinderpest-like reactions were observed in four breeds of cattle—Ayrshire, Guernsey, Jersey and Red Poll (Table 5). The incidence in Guernsey cattle was

significantly greater than in the other three breeds ($\chi^2 = 34{\cdot}418$, $P < 0{\cdot}001$). The animal which died was a Guernsey.

The ratio between herds with and without reactors gives a more realistic picture of the incidence of rinderpest-like reactions. Reactors occurred in 2 out of 177 Ayrshire herds (1 %), in 6 out of 151 Guernsey herds (4 %), in 2 out of 75 Jersey herds (3 %) and in 2 out of 63 Red Poll herds (3 %). Two of the 6 reacting Guernsey herds accounted for 47 and 61 reactors respectively or, in other words, 75 % of the Guernsey cattle that reacted.

Table 4. *The breed incidence of adverse reactions in cattle after vaccination with lapinized rinderpest virus*

Breed	No. of cattle vaccinated	Number of reactors: Rinderpest-like reactions	Aggravations of protozoal infections	Transient spermatolysis	Anaphylaxis
Aberdeen Angus	787	0	0	0	1
Ayrshire	40,302	20	24	0	0
Friesian	30,444	0	10	0	0
Galloway	81	0	0	0	0
Guernsey	26,837	144	11	0	0
Hereford	2,923	0	3	0	0
Jersey	9,796	21	3	0	0
Red Poll	15,020	32	1	6	0
Sahiwal	2,332	0	0	0	2
Shorthorn	5,193	0	0	0	0
South Devon	62	0	0	0	0
Sussex	24	0	0	0	0
Swiss Brown	186	0	0	0	0
Zebu	85,455	0	0	0	0
Unclassified	91,529	0	0	0	0

Table 5. *Incidence rates of adverse reactions in different breeds of cattle vaccinated with lapinized rinderpest virus*

Breed	Percentage incidence: Rinderpest-like reactions	Aggravations of latent disease
Ayrshire	0·05	0·06
Friesian	0	0·03
Guernsey	0·54	0·04
Hereford	0	0·10
Jersey	0·21	0·03
Red Poll	0·21	0·01

Aggravation of latent protozoal infections occurred in Ayrshire, Friesian, Guernsey, Hereford, Jersey and Red Poll cattle. The incidence was highest in Hereford cattle and lowest in Red Polls but the difference cannot be tested for significance because of the small numbers of cattle involved within each breed (Table 5). Fourteen (58 %) of the Ayrshire reactors, 5 (45 %) of the Guernsey reactors and the only Red Poll reactor died. The Friesian, Hereford and Jersey reactors survived.

The transient spermatolysis occurred in Red Poll bulls. The anaphylactic reactions appeared in Aberdeen-Angus and Sahiwal cattle.

Familial incidence

The incidence of rinderpest-like reactions within reacting herds was $17{\cdot}0 \pm 8{\cdot}1$ %. The rinderpest-like reactors within a herd were always related, usually through the sire. Amongst Guernsey cattle, nearly 80 % of the rinderpest-like reactors occurred in one blood line.

Significant blood relationships were not detected in cattle with aggravated protozoal infections, nor in the bulls with transient spermatolysis.

Two of the cattle which exhibited anaphylaxis were related, the one being the dam of the other. The younger was only two weeks old and its response was considered to be due to passively transferred sensitization.

Sex incidence

Six of the 217 rinderpest-like reactors were bulls, giving a ratio of 1/36. The Kenya Agricultural Census, 1954, recorded a sex ratio of 1/33 (Anon, 1955). Sex therefore probably did not influence the incidence. Likewise sex did not influence the incidence of aggravated latent infections. A 'flare-up' was observed in one vaccinated bull. The remainder occurred in female stock. Two of the 3 cattle which developed anaphylaxis were females.

Age incidence

Ninety-nine of the rinderpest-like reactors were less than 18 months of age and 118 were older. The ratio was similar to that noted in the Kenya Agricultural Census, 1954 (Anon, 1955). In other words, differences in innate resistance due to age were not evident. Similarly, age did not influence the incidence of aggravated latent infections. The cattle exhibiting anaphylaxis were 2 weeks, 3 years and 10 years old, respectively.

Immunological status of reactors

Rinderpest-like reactions occurred only in previously unvaccinated cattle. In Kenya outbreaks of rinderpest are now infrequent. Nevertheless, most cattle are immune through vaccination and only one-third of the animals presented for vaccination are estimated to be previously unvaccinated. If these are the cattle at risk, the percentage incidence rates of rinderpest-like reactors in Ayrshire, Guernsey, Jersey and Red Poll cattle are increased to 0·15, 1·61, 0·64 and 0·64, respectively (Table 6).

Fifty-one of the cattle in which lapinized rinderpest virus aggravated latent infections were previously unvaccinated. The remaining animal was an adult high grade Guernsey which had been vaccinated with lapinized rinderpest virus $2\frac{1}{2}$ years before and was therefore probably susceptible when revaccinated. If susceptible cattle are the true cattle at risk, the incidence rates of aggravated latent infection are increased to a high of 0·3 % in Herefords and to a low of 0·02 % in Red Polls (Table 6).

The transient spermatolysis occurred in rinderpest-susceptible bulls.

Two of the cattle exhibiting anaphylaxis were vaccinated for the first time. The third animal had been vaccinated annually for several years and during the last 3 years the vaccine had induced anaphylaxis.

Table 6. *Estimated incidence rates of adverse post-vaccinal reactions in different breeds of previously unvaccinated cattle*

Breed	Percentage incidence	
	Rinderpest-like reactions	Aggravations of latent disease
Ayrshire	0·15	0·18
Friesian	0	0·10
Guernsey	1·61	0·12
Hereford	0	0·31
Jersey	0·64	0·09
Red Poll	0·64	0·02

Table 7. *Batches of lapinized rinderpest vaccine which induced rinderpest-like reactions in cattle*

Batch Number	Number of cattle vaccinated	Number of rinderpest-like reactors
387/54	3,570	47
400/54	14,250	52
411/54	7,262	7
413/54	8,640	20
18/55	10,500	61
19/55	2,250	4
22/55	7,500	10
24/56	12,900	13
29/56	9,375	3

Retests of vaccine batches

Nine out of 109 batches of lapinized rinderpest vaccine used in Kenya in the period under review evoked rinderpest-like reactions in cattle (Table 7). Ampoules of the first four batches were retested in rabbits and cattle. The results agreed closely with those of the original batch tests and there was no evidence that the vaccine differed from normal lapinized rinderpest virus. Further retests were regarded therefore as unnecessary.

Recovery of virus from reacting cattle

Seventeen attempts were made to isolate virus from cattle with rinderpest-like reactions. All attempts from samples sent to the laboratory failed. Four of the seven attempts to isolate virus by bleeding the reacting cattle and immediately inoculating the blood into rabbits brought to the farm succeeded. One isolate was

from an Ayrshire (A), one came from a Jersey (J), and two were from Guernsey cattle (G 1, G 2).

Suspensions of tissues from the infected rabbits were inoculated subcutaneously into cattle which were housed along with uninoculated susceptible cattle. Neither the 16 inoculated cattle nor the 16 uninoculated controls exhibited a clinical response and there was no evidence that the isolated viruses were virulent. The cattle were challenged 14 days later by the injection of 10,000 CID_{50} virulent rinderpest virus. The inoculated group proved to be immune whereas the control cattle were all susceptible and 12 died.

In other words, the four isolated viruses were genotypically 'normal' lapinized rinderpest viruses in that they infected and induced pathognomonic lesions in rabbits; they infected grade cattle without stimulating a clinical reaction yet induced immunity to virulent rinderpest virus; and they were non-contagious.

DISCUSSION

Adverse reactions in Kenya cattle after vaccination with lapinized rinderpest virus fell into four categories: rinderpest-like, aggravation of latent protozoal infections, transient spermatolysis and anaphylaxis. The most serious were the aggravations of latent infections because of the attendant high case mortality rate, which approached 40 %. The commonest were rinderpest-like reactions which were without serious sequelae except in pregnant animals.

The over-all incidence of adverse reactions was 9 per 10,000 cattle vaccinated, which compares favourably with the incidence of adverse reactions in domestic animals and man given other attenuated virus vaccines. However, the total number of cattle vaccinated was not the true population at risk. The first three categories occurred only in animals which were being vaccinated for the first time and were directly attributable to the effects of virus multiplication. Accordingly the true population at risk was confined to rinderpest-susceptible cattle, which were estimated as being one-third of those presented for vaccination. Moreover, adverse reactions were not evident in *Bos indicus* cattle. The real incidence rate therefore approaches 3 per 1000 cattle at risk. This figure can be validly compared with the 95 % incidence in zebu cattle given caprinized vaccine (Daubney, 1949*b*) because expected post-caprinized reactions would be classified in this paper as adverse. In fact, adverse reactions associated with caprinized vaccinations are measured by the case mortality rate, which is 2 % in zebu cattle and up to 7 % in European breeds. The case mortality rate in cattle with adverse reactions after vaccination with lapinized rinderpest virus was nil in zebu cattle and 7·2 % in European breeds. The similarity in the figures is misleading because the caprinized vaccine case mortality rate is virtually synonymous with the over-all mortality rate. The lapinized vaccine case mortality rate differed dramatically from the overall mortality rate of 0·006 % of cattle vaccinated or 0·05 % of cattle at risk. Lapinized rinderpest vaccine was therefore safer than caprinized vaccine.

The only serious sequel to rinderpest-like reactions was abortion. Unfortunately the significance of the pregnant animal was realized too late and the over-all

abortion rate in pregnant animals could not be determined. Nevertheless, it would appear to be advisable to ensure that primary vaccination is carried out when animals are immature. Susceptible pregnant animals should not be vaccinated.

The sequel to a simple interaction between a virus and a susceptible host is largely governed by two factors, the virulence of the virus and the innate resistance of the host. The virulence of a virus for a particular host is probably a genetic characteristic (Burnet, 1955) and serial passage of a virus in a foreign host selects spontaneously occurring mutant genotypes which are avirulent for the natural host. The selection is fortuitous. For example, Koch (1897) passaged rinderpest virus simultaneously in goats and sheep. In goats, a strain slightly avirulent for cattle developed, but in sheep the strain developed an enhanced virulence for cattle. Edwards (1928) rediscovered the phenomenon of attenuation by serial passage in goats and thus pioneered the present types of rinderpest virus vaccines. Conversely Minett (1940) was unable to select an avirulent genotype despite more than 175 serial passages in goats. The attenuated rinderpest viruses used today as vaccines are considered 'fixed' and virulence for cattle is not regained despite serial passages in cattle (Nakamura, Fukusho & Kuroda, 1943; Waddington, 1945). Nevertheless, Ramon (1956) cited the Kenya incident reported by Brown *et al.* (1955) to support his argument that living virus vaccines should not be used. He postulated that such vaccines created carriers, and reactors to the virus caused new foci of infection and thus propagated the disease instead of assuring its prophylaxis. In other words, the stability of the virulence of the virus was variable. Our findings refute Ramon's conjecture, because the viruses which we recovered from reacting cattle were all genotypically lapinized rinderpest viruses.

The evidence strongly suggests that the true variable was the innate resistance of the cattle. Early workers recognized differences in innate resistance to rinderpest between local races or breeds of cattle (Seifman, 1866; Varnell & Pritchard, 1866) and since then the phenomenon has been well documented from all parts of the world. The differences were not correlated with the species. As a generalization we accept the current hypothesis regarding variations in innate resistance of cattle to rinderpest first mooted by Mornet (1948), and which was expressed by Henning (1949) as an evolution by a process of natural selection from ancestors which had survived previous visitations of the disease; a hypothesis which fulfils Theobald Smith's concept (1934) of the host-parasite relationship's equilibrating to the mutual advantage of both the parasite and the host. East African short horned zebus possess a marked innate resistance to rinderpest, and cattle of European origin have a low innate resistance (Cornell & Evans, 1937). This difference was clearly revealed in the survey but in addition shades of innate resistance were found within the non-resistant group. The important factor governing the degree of resistance appeared to be familial, because rinderpest-like reactions tended to occur in related cattle. Neither the sex nor the age of the cattle influenced the incidence of reactions.

When the virus–host interaction was complicated by the presence of a latent protozoal infection the sequel was serious. Aggravation of a protozoal infection occurred only in rinderpest-susceptible stock and therefore would appear to be a

direct result of virus multiplication. The phenomenon is familiar to older veterinarians who used the serum-virus simultaneous method for protecting cattle against rinderpest and Mornet, Gilbert, Orue & Thiery (1955) have also observed 'flare-ups' in cattle vaccinated with lapinized rinderpest virus in French West Africa. The mechanism has never been explained.

SUMMARY

A survey embracing all cattle vaccinated in Kenya with lapinized rinderpest virus between 1 January 1954 and 30 June 1956 revealed an over-all incidence rate of adverse reactions of 0·09 %. The real incidence rate approached 3 per 1000 cattle because only susceptible cattle were at risk. Four types of reactions were recognized. The most serious, because of the attendant high case mortality rate, was aggravation of latent protozoal infections. The commonest reactions were rinderpest-like without serious sequelae except in pregnant cows, many of which aborted. Transient spermatolysis was observed in six vaccinated bulls and anaphylaxis was recorded rarely.

The incidence of rinderpest-like reactions was primarily influenced by the innate resistance of the cattle. The viral strains recovered from reacting cattle were genotypically unchanged.

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Studies in tissue culture on the pH-stability of rinderpest virus

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(*Received* 23 *January* 1963)

INTRODUCTION

Although information on the pH-stability of rinderpest virus has considerable practical importance and a comparative interest for those working with the measles–rinderpest–distemper group of viruses, we know of no adequate published data on this subject. An early report by Maurer (1946) is concerned with the stability of rinderpest virus, which had previously undergone several alternating calf-rabbit passages. The source of virus was spleen tissue from an infected calf and 1 % (w/v) suspensions in phosphate buffers of pH 6·0, 7·0 and 8·0 were maintained at 36° F. Infectivity was demonstrated by inoculating rabbits and recording the characteristic pyrexia which followed successful infection.

The introduction of tissue culture techniques for the propagation and titration of rinderpest virus *in vitro* has provided a cheaper, more accurate system for investigations of this problem. The results of our experiments with a tissue culture system are presented in the following report.

MATERIALS AND METHODS

Virus strains

In an initial experiment the 95th bovine kidney passage of the Kabete 'O' strain of rinderpest virus (RBOK) was employed. Culture fluids were harvested 7 days after seeding an infected cell suspension into 20 oz. medical flat bottles (Plowright & Ferris, 1959). They were clarified by light centrifugation (2000 r.p.m. for 5 min.) and stored at -70° C. The maintenance medium was that designated LA:YE by Franklin, Rubin & Davis (1957), with the addition of 5% normal, unheated ox serum.

In subsequent experiments two virulent field strains of virus were used, in addition to the RBOK strain which is highly attenuated. The origin of the RBT/1 isolate has already been described (Plowright & Ferris, 1962); the material employed consisted of a 9-day fluid harvest from its 3rd passage in bovine kidney cells. The third virus strain, RGK/1, was isolated in 1962 from lymph node tissue of a sick giraffe which was shot in the Northern Frontier District of Kenya. It produces an 80 % mortality in experimental cattle and very high levels of virus can be demonstrated in the tissues of sick animals by direct inoculation of tissue cultures. The RGK/1 strain was used for pH studies as a 10-day fluid harvest from the 2nd culture passage.

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Buffer solutions

The isotonic veronal-acetate buffers of Michaelis (Hull, 1943) were used throughout. Solutions ranging from pH 2·6 to 9·0 were prepared according to the original formulae. For pH 10·0 and 10·7, which were not covered in the original publication, adjustment from pH 9·6 was effected with 0·1 N or 1 N sodium hydroxide. The final pH was checked in each instance by using a meter with a glass electrode and thermal control element.

With the exception of the first experiment highly alkaline or very acid virus-buffer mixtures were adjusted to pH 7·6, at the end of the period of exposure; for this purpose an equal volume of a suitable complementary buffer was added.

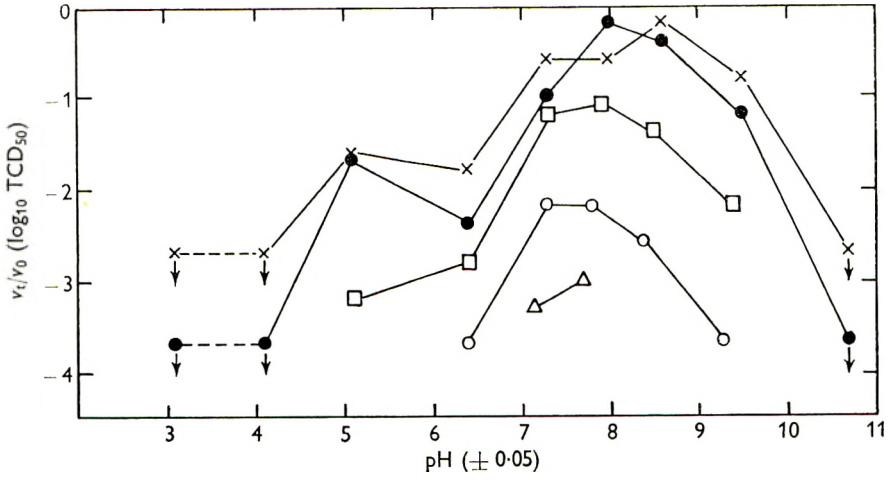

Fig. 1. Inactivation of rinderpest virus (strain RBOK) in culture fluids diluted tenfold in Michaelis's buffers at 4° C. Exposure: ×, 1 week; ●, 2 weeks; □, 3 weeks; ○, 6 weeks; △, 7 weeks.

Virus exposure and infectivity titrations

In the first experiment, designed to determine the rate of virus inactivation over a long period, culture fluid infected with the strain RBOK was diluted 1:10 in buffers to give a final pH of 3·1 to 10·7 (Fig. 1). Virus-buffer mixtures were kept in tightly stoppered, screw-capped bottles and the pH was checked on the occasion of each sampling. Minor changes of pH were noted and not readjusted but the fall in pH levels higher than 7·0 was taken into account in preparing Fig. 1. Below pH 7·0 no change of more than 0·05 units could be detected over the test period of 7 weeks.

Judging from the results of the initial experiment a rapid fall of infectivity was not to be expected except between pH 3·0 and 5·0 and between pH 9·5 and 11·0. Subsequent experiments within these ranges were therefore designed with short exposure times, ranging from seconds to not more than 24 hours. The more refined technique of Bachrach *et al.* (1957) was adopted to ensure accuracy of exposure. Virus preparations were added (1:10, v/v) to buffers of the required pH, using a magnetic stirrer to ensure instantaneous and uniform distribution of the virus. At suitable intervals aliquots of the virus-buffer mixtures were withdrawn and

added to an equal volume of the required neutralizing buffer. All buffers and virus-buffer mixtures were precooled and kept at 4° C. throughout the duration of all except the shortest experiments.

Virus assay was carried out by inoculating serial tenfold dilutions in culture growth medium into tubes containing trypsin-dispersed calf kidney cells (Plowright & Ferris, 1959) or into established cultures of the same cell type (Plowright & Ferris, 1962). Five tubes were inoculated per dilution and titres were expressed as $\log_{10}$ TCD 50 per ml. calculated by the method of Thompson (1947).

RESULTS

The first experiment was designed to show the effect of a wide range of pH values (3·1–10·7), on the inactivation of rinderpest virus over a period of 7 weeks at 4° C. There was no necessity to neutralize very acid or alkaline suspensions prior to inoculating them undiluted into culture tubes. The buffering capacity of 1 ml. of growth medium was sufficient to prevent any detectable effects of the virus inoculum on the growth potential of the cells.

The results of this experiment are expressed graphically in Fig. 1, which shows the surviving virus fractions, relative to the titre of the original culture fluid. The virus was quite stable from pH 5·0 to 9·6 but it is clear that relatively rapid inactivation of the infectivity occurred outside this range. Further experiments were therefore carried out to determine the rate of inactivation in the two critical zones and the results are given in Table 1.

Table 1. *Half-life of attenuated and virulent strains of rinderpest virus diluted tenfold in Michaelis's Buffers at* 4° C.

	Virus strain		
pH (± 0·1)	RBOK	RGK/1	RBT/1
3	24·0 sec.	12·5 sec.	—
4	2·3 hr.	—	2·2 min.
5	25·0 hr.	4·1 hr.	42·3 min.
7·2	3·68 days*	—	—
7·8–7·9	3·72 days*	—	—
9	50·1 hr.	8·7 hr.	16·7 hr.
10·2	2·6 hr.	—	5·5 hr.
10·7	5·0 min.	8·3 min.	—

* Results from initial experiment only.

Figure 2 presents data for two strains at pH 3·0. There were clear indications that the rate of loss of infectivity was higher for the recently isolated, highly virulent strain (RGK/1) than for the RBOK strain at high passage level. The difference between the regression lines as judged by the covariance test (Snedecor, 1959) was significant and the half-life periods were 12·5 and 24 sec., respectively (Table 1). It is also of interest to note that the inactivation rate at pH 4·0 and 5·0 for the other recent isolate, RBT/1, was considerably higher than that for the RBOK strain.

Figure 3 shows that the strains RBOK and RGK/1 were considerably less sensitive to exposure at pH 10·7 than at pH 3. It was barely possible to demonstrate a decline of titre over a period of 5 min. for the strain RGK/1.

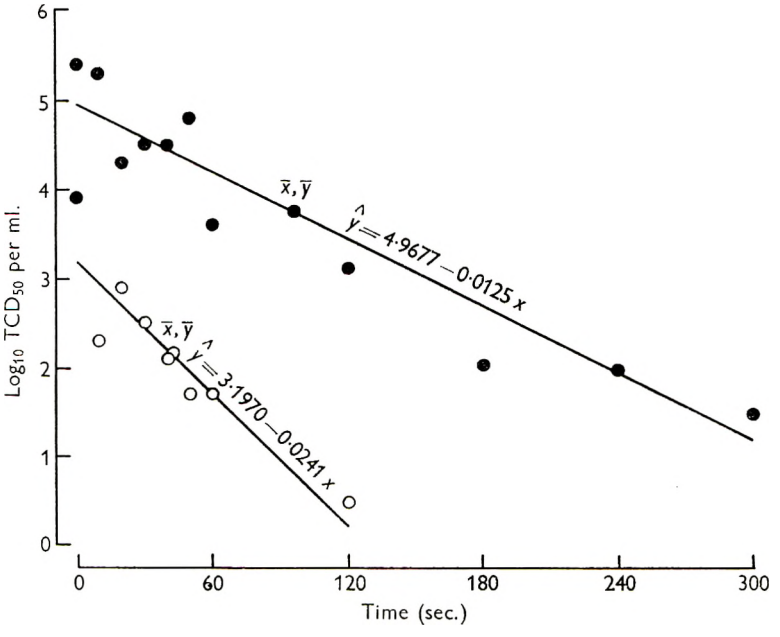

Fig. 2. Inactivation of virulent and attenuated strains of rinderpest virus at pH 3 and at 4° C. ●, Attenuated strain, Kabete 'O', 95 passages in bovine kidney cells; ○, virulent strain, RGK/1, 2 passages in bovine kidney cells.

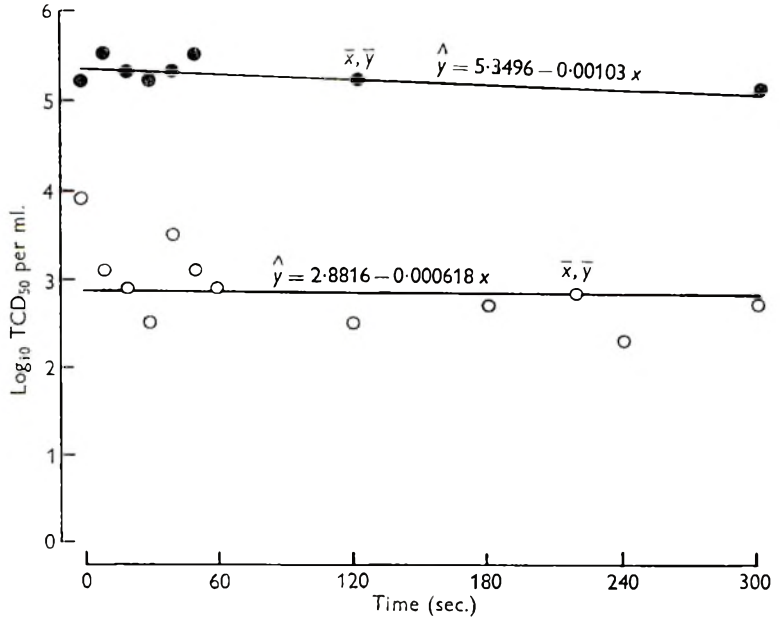

Fig. 3. Inactivation of virulent and attenuated strains of rinderpest virus at pH 10·7 and at 4° C. ●, Attenuated strain, Kabete 'O', 95 passages in bovine kidney cells; ○, virulent strain, RGK/1, 2 passages in bovine kidney cells.

DISCUSSION

There appear to be no generally accepted criteria in assessing the pH stability of viruses and much of the published information has been obtained in experiments which failed to determine the initial concentration of virus or the rate of inactivation, as opposed to the mere presence or absence of infectivity. Other variables which need to be taken into consideration are the composition of the suspending medium, the origin and passage history (Gorham, 1960) of the virus isolate, and the temperature of exposure. Variations in the procedures which have been employed are matched in numbers by the methods used to express the results. In this respect there can be little doubt that the most useful abstraction is the half-life period derived from the regression coefficient, calculated from an adequate series of observations over a significant period of time for each pH value.

The utility of the half-life period will be limited, of course, if a virus population is heterogeneous with respect to its pH stability, as shown for example by Bachrach *et al.* (1957) for foot-and-mouth disease virus at pH 5 and 6. We did not obtain any evidence that rinderpest virus exhibits any such heterogeneity.

The effect of the suspending medium, at least so far as long term experiments are concerned, was shown by a comparison of results in this study with those obtained by Plowright & Ferris (1961). These authors found that the mean half-life at 4° C. of virus in the form of undiluted culture fluids was 9·2 days in four experiments with virus of the strain RBOK in its 39th to 97th culture passages; the pH of these fluids varied from approximately 7·3 to 7·6 and they contained 5 % ox serum. Similar fluids diluted tenfold in Michaelis buffer, i.e. with 0·5 % serum and a tenfold reduction in other medium constituents, had a half-life in the present experiments of 3·72 days at pH 7·7–8·0 and 3·68 days at pH 7·2–7·3.

The differences between the pH stability of the three strains of rinderpest virus employed here illustrate the dangers of generalizations from experience limited to a single isolate. The strain RBOK, for example, was relatively stable at pH 5·0 and 9·0 and a significant fall of titre would not be expected over a 24-hr. exposure period (half-life periods of 25·0 and 50·1 hr., respectively). The isolate RBT/1, on the other hand, was unstable at pH 5·0, with a half-life of 42·3 min., but more stable than the strain RBOK at pH 10·1 (half-life 5·5 hr., compared with 2·6 hr.). As already noted, RGK/1 virus had a somewhat lower stability than RBOK virus at pH 3·0 but was at least equally stable in the higher pH range up to 10·7. These differences may be accounted for by selection pressure or mutation within the RBOK virus population during prolonged culture passage; as described in the section on Materials and Methods the other two strains were in their second and third culture generations only, following isolation from field outbreaks of the disease.

Maurer (1946) concluded that the optimal pH for survival of his strain of rinderpest virus lay between 6·5 and 7·0, using M/10 phosphate buffers. The results recorded here clearly indicate that the optimal pH for the RBOK strain of virus was between 7·5 and 8·0. A detailed comparison of the two studies is not possible, however, since Maurer neither stated the initial titre of his preparation nor the number of rabbits used for each aliquot.

Of the other members of the measles–rinderpest–distemper group (Warren, 1960), the pH-stability of measles virus was investigated by Black (1959) and Black, Reissig & Melnick (1959) who reported complete inactivation within 3 hr. at 0° C. and pH 4·4 or below; there was little loss of infectivity between pH 6·0 and 10·5 but no point between pH 6·0 and 4·4 was investigated. The initial titre in this experiment was about $10^{3·5}$ p.f.u./ml. Essentially similar results were reported by Musser & Underwood (1960), but they found complete inactivation of a comparable virus preparation within 3 hr. at pH 10·0 and at 25° C. The optimal pH for virus survival was 7·0–8·0. The virulent rinderpest strains, RGK/1 and RBT/1, were shown to have a wider range of relative pH-stability than measles virus since they would not have shown a significant depression of titre over a period of 3 hr. at 4° C., if exposed to buffers of pH 5·0–10·2 (see Table 1). Similarly samples with an initial titre of $10^{3·5}$ would not have been completely inactivated by a 3-hr. treatment at pH 4·0 and 10·7, since the half-life under these conditions varied between 1·5 and 4·6 hr. The attenuated strain (RBOK) showed an even higher resistance at pH 4, with a half-life of 7·3 hr.

Bindrich (1951) investigated the pH-stability of a virulent strain of canine distemper virus in the form of infected serum and cellular washings from the blood of dogs. He diluted the virus in phosphate buffers of pH 3·0–12·0 and exposed the mixtures for 20 or 30 min. in the refrigerator. Infectivity was detected by inoculation of dogs and he concluded that the virus was relatively stable in acid media down to pH 3·6, but partially or completely inactivated at pH levels of 9·0 or higher. Celiker & Gillespie (1954) showed that the Onderstepoort strain of avianised distemper virus, as a suspension of infected chorio-allantoic membranes, survived 24 hr. treatment at 4° C. in the pH range of 4·4–10·4; it was inactivated within 2 hr. at pH 4·2 or 10·9.

In conclusion, it appears that all members of the measles–rinderpest–distemper group of viruses show a relatively high stability at pH 5–10. This, for example, may be compared with the different stabilities of small RNA viruses—high in the case of polioviruses (Bachrach & Schwerdt, 1952) and low in the case of foot-and-mouth disease (Bachrach *et al.* 1957).

SUMMARY

The pH-stability of three strains of rinderpest virus, propagated in tissue cultures, was investigated at 4° C. in veronal-acetate buffers (Michaelis) of pH 3·0–10·7.

An attenuated laboratory strain, in its 95th culture passage (RBOK), showed maximal stability in the pH range 7·2–8·0, the half-life being about 3·7 days. It was relatively stable from pH 4·0 to 10·2, the half-life at the extremes of this range being over 2 hr. At pH 3·0 the infectivity declined very rapidly, the half-life period being 24·0 sec.

One virulent strain (RGK/1) showed a significantly lower resistance than strain RBOK at pH 3·0, probably also at pH 5·0. The inactivation rates for these two strains were, however, not greatly different at pH 10·7. The other virulent strain (RBT/1) was even less stable than RGK/1 at pH 4·0 or 5·0 but of a comparable stability at pH 9·0 and 10·2.

These findings are discussed in comparison with published data on the related viruses of measles and canine distemper. The importance of strain differences in studies of this kind is stressed.

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The incidence of natural *Clostridium welchii* α-antitoxin in Indian equines: its influence on the results of antigenic stimulus

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(*Received* 1 *January* 1963)

The presence of natural circulating antitoxins in horses and its influence on hyperimmunization has been studied by many observers including Bolton (1896), Glenny (1925*a*, *b*), Barr & Glenny (1945), Basu & Roy (1946) and Ottensooser (1946). Bolton (1896), working with 2 horses, failed to observe any correlation between the amount of natural circulating antitoxin and the results of immunization with diphtheria toxin. Other observers mentioned above, working with a much larger number of animals and with different exotoxins, obtained a definite relationship between natural immunity and the immunological performances of the animals on subsequent hyperimmunization, and the significance of the presence of natural antitoxin is now well recognized.

EXPERIMENTAL

In the present study, natural circulating *Clostridium welchii* α-antitoxin in 65 young Indian equines—15 horses and 50 mules—was titrated by the lecithovitellin method. Only a single test was done for each animal, shortly after it arrived from north-western parts of India. The mules were from the sub-Himalayan regions and had previously been used as pack and draught animals in hills and towns, while the horses belonged to towns and cities. The end titre in 6 of the mules was not determined but they all contained 1 unit or more per ml. of serum. The titres (units/ml.) observed in the remaining 59 animals are shown in Table 1.

Only 7 of these 59 animals were subsequently immunized for production of *Cl. welchii* α-antitoxin, and these yielded antitoxin of potency shown in Table 2.

Table 1

	Total number	Number of animals			
		1–2 units	2–3 units	3–4 units	4–5 units
Horses	15	2	6	5	2
Mules	44	2	24	15	3
Total	59	4 (7 %)	30 (51 %)	20 (34 %)	5 (8 %)

Titres determined by lecithovitellin reaction.

Table 2

Serial number of equine	Natural titre (units/ml.)	Titre after immunization (units/ml.)
2026	2	500
2030	4	307
2038	4	307
2039	1	250
2040	1	500
2050	1	166
2062	3	500

Titres determined by lecithovitellin reaction.

DISCUSSION

Barr & Glenny (1945) working with a larger number of horses observed that natural Staphylococcus α-antitoxin was present in all of them, while only a small proportion of animals had any detectable diphtheria and *Cl. welchii* α-antitoxins Ottensooser (1946) observed only 20% of horses in São Paulo had 0·04 unit or more of *Cl. welchii* α-antitoxin per ml. of serum.

In the present series of 65 equines every animal had 1 unit or more per ml. naturally present *Cl. welchii* α-antitoxin and about 8% of the animals had 4 units/ml. (Table 1). These figures are very much higher than the figures obtained by previous workers quoted above (Barr & Glenny, 1945; Ottensooser, 1946). Variations in degree and frequency of natural immunity present in animals of different geographical origin have previously been observed (Glenny, 1925*a*, *b*; Barr & Glenny, 1945; Basu & Roy, 1946). These variations are explained by the difference in environmental circumstances and frequency of natural infection. A cause of such high incidence of natural *Cl. welchii* α-antitoxin in the present series of Indian animals may also lie in their very special environmental condition, and frequent natural infection.

Antitoxin level in a random sample of natural serum depends on several factors, namely, frequency of and intervals between previous contacts of the animal with the particular antigen, interval between contact and drawing of serum sample for test, and inherent sensitivity of the antibody-producing cells of the particular animal. A single determination of natural immunity cannot reveal fully the immunological possibilities of an animal. Performance test—i.e. response to preliminary antigenic stimulus—is likely to demonstrate potential immunity more correctly. That in the limited number of animals hyperimmunized with *Cl. welchii* α-toxin the titres obtained were not directly proportional to the natural immunity observed in each of them (Table 2), is, as such, not difficult to explain and is in conformity with previous observations (Barr & Glenny, 1945; Basu & Roy, 1946).

SUMMARY

Presence of natural *Cl. welchii* α-antitoxin in 65 Indian equines was observed. All the 65 equines had 1 unit or more of circulating natural antitoxin per ml. of serum. The maximum titre observed was 4 units/ml. in about 8 % of the animals. These figures are very much higher than the figures obtained in other countries. Such a high incidence of natural immunity indicates frequent natural contact with the antigen concerned.

Results of hyperimmunization of 7 equines are recorded and discussed.

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Antigenic differences between strains of foot-and-mouth disease virus of type SAT 1

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INTRODUCTION

Strains of foot-and-mouth disease (F.M.D.) virus of types O, A and C, isolated during the past 15 years from outbreaks of the disease in the field, have exhibited marked differences of invasiveness, of virulence and of pathogenicity for various animal species. Tests *in vitro* reveal that many strains show preferential fixation of complement in the presence of antiserum against the identical strain, and a quantitatively inferior degree of fixation with antisera produced by other strains of the same type. Furthermore, there is now considerable experimental evidence that differences in antigenic constitution can be demonstrated not only by serological methods, complement fixation and serum neutralization tests, but also by cross immunity experiments in cattle.

Traub & Möhlmann (1946) and Galloway, Henderson & Brooksby (1948), in studies of type A strains, were the first to demonstrate the significance, in relation to immunization, of antigenic differences between strains within a main immunological type by serological tests and experimental cross immunity tests in cattle.

From 1947 onwards the investigators at Pirbright have made comparisons of eight pairs of strains belonging to four different type groups—O, A, SAT 1 and SAT 2—by complement fixation and serum neutralization tests and by cross vaccination experiments in cattle.

Type A strains: 119 and MP; 119 and MI; MI and MP (referred to above, Galloway *et al.* 1948)).

Type O strains: Ven 1 (Venezuela 1950) and H_2 (Holland). H_{2200} (Holland 1951) and Ca 1 (Canada 1952). H_{2200} and 734 (Great Britain 1951).

Type SAT 1 strains: RV 11 (Southern Rhodesia 1937) and Bec 1 (Bechuanaland 1948).

Type SAT 2 strains: Rho 1 (Northern Rhodesia 1948) and RV 1 (Southern Rhodesia 1931).

The results obtained with three of the pairs—Rho 1 and RV 1, H_{2200} and Ca 1, H_{2200} and 734—indicated that the two strains compared in each case were identical in their antigenic behaviour. With the other five pairs, the results indicated that the strains in each pair differed in their antigenic structure and could be classified as variant strains (subtypes). In the case of the similar pairs, a vaccine prepared from one strain protected quantitatively equally well against infection with either strain, whereas, in the case of dissimilar pairs, a vaccine prepared from one strain

protected better against the homologous strain than against the heterologous strain.

Martin, Davies & Smith (1962) found that a mouse-adapted vaccine strain (Rho 1/48) of type SAT 2 evoked good protection against challenge with the homologous strain but only incomplete protection against challenge with the heterologous strain SA 106/59. Complement fixation (C.F.) tests indicate that these strains are of different subtypes (Davie, 1962).

Girard & Mackowiak (1950) reported strain differences in strains of type O. Ubertini (1951) recorded similar observations in investigations on strains of type A and O recovered from outbreaks in the Po valley. These authors stressed likewise that demonstrable differences in antigenic structure of strains of the same immunological type were correlated with the results of vaccination, though Röhrer, Möhlmann & Pyl (1951) considered these differences to be of little practical importance.

Although antigenic differences between strains of type C and type SAT 3 have been detected by serological methods, cross-immunity tests with these strains have not been made. As regards type ASIA 1 no information is available as yet on subtype differences between strains. However, there is no reason to suppose that the results of investigations on strains of these three types would be at variance with those reported on the other four main immunological type-groups.

A recent outbreak of F.M.D. in South-West Africa gave impetus to investigations on several strains of type SAT 1. The following report demonstrates the effect of immunological differences between two strains which were revealed by cross protection tests *in vivo* and confirmed *in vitro* by C.F. tests and by neutralization of virus with serum from immunized cattle. The paper also indicates that it is possible to prepare potent inactivated vaccines against F.M.D. virus of type SAT 1.

MATERIALS AND METHODS

Virus

Type SAT 1 F.M.D. virus of strain RV 11, isolated in 1937 from an infected kudu in Southern Rhodesia, and of strain SA 13/61, recently isolated from an ox during an outbreak in the Northern Transvaal, were each grown in tongue epithelium tissue cultures and used for the production of formalin-inactivated vaccines. Virulent virus of both strains, serially passaged in susceptible cattle, was employed to challenge the resistance of vaccinated animals. Guinea-pig adapted strains of the same isolates were maintained for similar tests in guinea-pigs.

Cattle

All cattle used in these experiments were Devon steers, about 18 months old, which were purchased through a dealer who collected them from various parts of south-west England. During the experiments all cattle were housed in isolation.

Guinea-pigs

Groups of young guinea-pigs (about 500 g. body weight) were obtained from the small-animal breeding unit at this Institute. Guinea-pigs infected with different strains were housed in separate rooms.

Mice

Infectivity titrations were made by inoculating serial dilutions of virus suspensions into 4–6-day-old albino mice of the Pirbright 'P' strain.

Vaccine production

The method employed at Pirbright is a modification of that described by Frenkel (1949, 1951, 1953), virus being propagated, inactivated and stored in large mechanically stirred stainless steel vessels, the temperature of which is accurately determined by thermostatically controlled water-jackets.

In the production of a typical batch of vaccine, 7·5 kg. of freshly harvested bovine tongue epithelium fragments were coarsely minced and then suspended in 200 l. of a medium consisting of Tyrode's solution containing 0·5 % lactalbumin hydrolysate and antibiotics (Penicillin, Neomycin and Mycostatin).

'Seed' virus was then inoculated and the culture was incubated for 16 hr. at 37 °C., pH being maintained at about 7·6 by aeration with a mixture of 5 % CO_2/95 % O_2 (7·5 l./min.).

Tissue fragments were then strained from the fluid, milled into a paste and resuspended in the culture fluid to extract residual virus. After clarification by continuous-flow centrifugation, the extract was filtered through Seitz K and EK grade asbestos pads mounted in series, and was then pumped into jacketed vessels maintained at 26 °C. Samples were withdrawn for infectivity titrations in mice.

The suspension was mixed with 250 l. of aluminium hydroxide gel and 5 l. of 2 M glycine buffer (pH 9·0) and exposed to the action of 250 ml. of 40 % formaldehyde solution. Inactivation continued for 48 hr. at 26° C., after which the temperature was reduced to 4° C. to inhibit further action of any residual formalin.

Storage

Vaccine batches were bottled mechanically and stored in cold rooms at 4° C. Stock strains of virulent virus were stored either at −20° C. or at 4° C. in other refrigerators.

Vaccine assay

Innocuity. Volumes of 0·1 ml. from pooled samples of each batch of vaccine used in these experiments were inoculated into the lingual epithelium at 20 sites on the tongue of each of 6 susceptible steers; no F.M.D. lesions were observed. The validity of such tests was discussed by Henderson (1952*a*, *b*).

Potency. The potency of each batch of vaccine was determined by inoculating a group of steers with the usual 15·0 ml. dose of undiluted vaccine and a further group with the same volume of vaccine diluted 1/3 with inert vaccine base. After 3 weeks the cattle were challenged, together with unvaccinated controls, by inoculating 10,000 ID 50 of virulent cattle-passaged virus into the epithelium at each of 10 sites on the tongue. All animals were bled for serum before vaccination and again before challenge. Cattle which showed no secondary lesions at sites other than in the oral cavity were considered to be resistant to infection.

Samples of tongue epithelium collected from 'virus donor' cattle (in which the virus was passaged immediately before the challenge experiment), and also from representative reacting cattle in each group, were examined for virus specificity against all seven types of F.M.D. antiserum by the C.F. test.

Serum-neutralization tests

Pre-vaccination and pre-challenge samples of serum were titrated for neutralizing antibody by the tissue culture colour test technique described by Martin & Chapman (1961) with a slight modification. All sera were titrated in serial twofold dilutions against 100 T.C. ID50 of virus of each of the strains used in the cattle experiments; the usual controls were included in each test. Results were expressed as the final dilution in the serum-virus mixture.

Complement-fixation tests

Virus of each of the strains under investigation was adapted to growth on the plantar pads of guinea-pigs, and strain-specific hyperimmune sera were produced. Complement-fixation tests employing these sera followed the method described by Brooksby (1952) and the results were assessed in accordance with the principles described by Bradish, Brooksby & Tsubahara (1960) and by Bradish & Brooksby (1960).

Each virus was tested with each of the strain-specific sera, and the amount of complement required for 50% haemolysis was determined. The results were expressed as the cross-fixation ratios (C.F.R.) of the systems, i.e.

$$\frac{\text{amount of complement fixed in the heterologous system}}{\text{amount of complement fixed in the homologous system}}.$$

The product of the C.F.R. for two strains was used to indicate their degree of antigenic relationship.

RESULTS

1. *Tests in guinea-pigs*

A group of 10 guinea-pigs was infected by inoculation of the left plantar pad with guinea-pig passaged F.M.D. virus of strain RV 11. A similar group was infected with strain SA 13/61. The disease generalized in all animals. After a recovery period of 28 days, 5 guinea-pigs from each group and 4 uninoculated controls were inoculated on the right plantar pad with virus strain RV 11; the remaining animals of the convalescent groups and 4 susceptible controls were inoculated with strain SA 13/61 virus.

The results of this experiment, which are shown in Table 1, indicated that the antigenic constitutions of the two strains were dissimilar, severe infection with one strain stimulating good resistance to reinfection with the same strain but evoking only poor protection against the other strain.

Another cross-protection experiment (Table 2) demonstrated that intradermal inoculation of virulent suspensions of strain RV 11 on two occasions, with an interval of 19 days between, failed to protect 1 of 2 guinea-pigs reinoculated 22 days later with virus of strain SA 13/61.

2. *Tests in cattle*

Groups of cattle were inoculated with vaccine of batches 613 (RV 11), 614 and 615 (SA 13/61). The virus content of these vaccines, as determined by infectivity tests before inactivation, was $10^{6.8}$, $10^{6.9}$ and $10^{7.1}$ mouse ID50/ml., respectively.

Innocuity tests by inoculation into the epithelium of the tongue did not reveal infectivity in any of the batches, and all the cattle vaccinated by the subcutaneous route remained normal during the 3 weeks' period between vaccination and challenge.

Table 1. *Reinfection of guinea-pigs convalescent from experimental infection with* F.M.D. *virus of type SAT* 1

Recovered from	Reinfected with	Lesions	
		Primary	Generalized
RV 11	RV 11	2/5	0/5
SA 13/61	SA 13/61	0/5	0/5
SA 13/61	RV 11	5/5	2/5
RV 11	SA 13/61	5/5	3/5
Controls	RV 11	5/5	5/5
Controls	SA 13/61	5/5	5/5

Table 2. *Reinfection of guinea-pigs hyperimmunized with* F.M.D. *virus of type SAT* 1

Immunization strain	Guinea-pig lesions after challenge	
	RV 11	SA 13/61
RV 11	0/2	1/2
SA 13/61	0/2	0/2
Controls	2/2	2/2

(A) *Challenge with virus of the homologous strain*

In a group of 10 steers inoculated with undiluted vaccine of strain RV 11 (batch 613), none developed secondary lesions after challenge with 10^5 ID50 of virulent virus of the same strain. In a similar group inoculated with Batch 613 diluted 1/3 in aluminium hydroxide-buffer base, 3 showed some degree of generalization. Generalization to all feet occurred in 3 unvaccinated controls.

Further groups of 10 steers were vaccinated with undiluted vaccine of strain SA 13/61 from batches 614 and 615, and a 3rd group was inoculated with vaccine of batch 615 diluted 1/3. All animals which had received undiluted vaccine withstood the challenge without generalization of infection. Incomplete generalization occurred in 2 of the 10 steers which were inoculated with diluted vaccine. In contrast, lesions generalized in all of 10 unvaccinated controls.

Columns 8 and 16 of Figs. 1 and 2 show the clinical results for individual steers. The black symbol in the first square represents the extent of the primary lesions on the tongue, and a diagonal band in this square indicates development of vesicles

in parts of the buccal cavity other than the tongue (usually the lips, gums or dental pad). Black symbols in the succeeding squares represent lesions on 1 or more feet.

(*B*) *Challenge with virus of the heterologous strain*

The satisfactory results obtained with both strains, when the immunity of vaccinated cattle was challenged with the homologous strain, were not observed when cattle vaccinated with either strain were exposed to challenge with the heterologous strain.

Of 10 steers inoculated with undiluted vaccine of strain RV 11 and challenged 22 days later with strain SA 13/61, only 5 were protected completely. In the similar group for which the vaccine was diluted 1/3, none was protected completely although 2 developed lesions on the dental pad only (Fig. 1). Generalization was also complete in all of 6 unvaccinated steers.

The difference between strains RV 11 and SA 13/61 was revealed even more clearly when the immunity of cattle inoculated with strain SA 13/61 vaccine was challenged with virus of strain RV 11.

As in the corresponding experiment, in which the cattle were challenged with strain SA 13/61, 10 steers were inoculated with undiluted vaccine of batch 614, 10 steers received undiluted vaccine of Batch 615 and a further 10 steers were inoculated with vaccine of Batch 615 diluted 1/3. Among the groups which received undiluted vaccine a significant difference between vaccine batches was not observed, the strain RV 11 challenge virus generalizing to 1 or more feet in each case. Of the 10 cattle inoculated with vaccine diluted 1/3, all developed foot lesions. Infection generalized to all the feet of 4 unvaccinated control animals (Fig. 2).

To provide additional evidence of the identity of the challenge material, part of the virus filtrate used to infect the cattle was subjected to a C.F. test against reference antisera of Types O, A, C, SAT 1, SAT 2, SAT 3 and ASIA 1. The test was positive for SAT 1 only. Another part of the filtrate was inoculated into mice, and suspensions of the triturated carcasses of mice dying from these inoculations were pooled; samples from this pool were mixed with equal volumes of serial dilutions of SAT 1 antiserum before subinoculation into further mice. Carcasses of mice dying at the end-point dilution, when used as antigen for C.F. tests against the 7 reference antisera, produced a positive result with SAT 1 antiserum only.

3. *Serum neutralization tests*

The results shown in Figs. 1 and 2 indicate that there was an increase in virus-neutralizing antibody in the sera of the majority of cattle during the 3 weeks interval between vaccination and challenge.

For various reasons, pairs of serum samples collected before vaccination and before challenge were not available for all of the 100 vaccinated cattle. When samples were tested against virus of the strain incorporated in the vaccine, only 3 of the 97 animals for which pairs of sera were available failed to show an increase in titre. When the same sera were tested against virus of the alternative strain,

Challenge strain RV 11

Antigen strain RV 11			Serum titre				
Vaccine			Day 0		Day 22		
No.	Dilution	Animal No.	RV 11	13/61	RV 11	13/61	Lesions
613	0	DL 18	1/3	1/16	1/256	1/3	
		19	1/11	1/22	1/90	N.A.	
		20	1/11	1/22	1/45	1/11	
		21	1/8	N.A.	1/355	N.A.	
		22	1/3	1/3	1/90	1/22	
		23	1/11	1/22	1/64	N.A.	
		24	1/16	1/16	1/64	1/16	
		25	1/11	1/16	1/90	1/22	
		DD 26	1/3	1/3	1/64	1/22	
		27	N.A.	1/11	1/64	1/22	
613	1/3	DD 28	1/3	1/22	1/64	N.A.	
		29	1/3	1/8	1/90	1/22	
		30	1/16	1/3	1/128	1/22	
		31	1/16	1/3	1/45	N.A.	
		32	1/11	1/3	1/45	N.A.	
		33	1/16	1/3	1/45	N.A.	
		34	1/22	1/3	1/90	N.A.	
		35	1/22	1/3	1/90	1/45	
		36	1/22	1/3	1/22	1/22	
		37	1/3	1/3	1/45	1/16	
Unvaccinated controls		DL 26					
		27					
		28					

Challenge strain 13/61

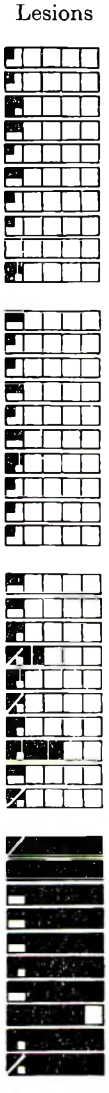
Antigen strain RV 11			Serum titre				
Vaccine			Day 0		Day 22		
No.	Dilution	Animal No.	RV 11	13/61	RV 11	13/61	Lesions
613	0	DK 72	1/8	1/3	1/90	1/45	
		73	1/3	1/3	1/45	N.A.	
		74	1/3	1/3	1/45	1/8	
		75	1/22	1/32	1/90	1/45	
		76	1/11	1/3	1/128	N.A.	
		77	1/8	1/3	1/90	1/11	
		78	1/11	1/8	1/90	1/45	
		79	1/22	1/11	1/178	1/22	
		80	1/11	1/3	1/178	1/45	
		81	1/8	1/8	1/128	1/22	
613	1/3	DK 82	1/11	1/3	1/32	1/11	
		83	1/8	1/4	1/64	1/11	
		84	1/8	1/11	1/90	1/22	
		85	1/8	1/3	1/178	N.A.	
		86	1/8	1/3	1/45	1/3	
		87	1/22	1/3	1/90	1/16	
		88	1/11	1/3	1/64	1/3	
		89	1/16	1/16	1/32	1/8	
		90	1/6	1/3	1/178	1/22	
		91	1/11	1/3	1/90	1/22	
Unvaccinated controls		DL 12					
		13					
		14					
		15					
		16					
		17					

Fig. 1. Neutralizing antibody responses of cattle to RV 11 antigen, and reactions to challenge after 22 days. N.A., Result not available.

24 of 87 pairs of sera did not show a rise in titre, and of these 6 showed a slight (but probably non-significant) fall.

Individual cattle showed well-marked differences in the response to the antigenic stimulus of the vaccines and, for this reason, groups of cattle will be considered as a whole. Table 3 shows the geometric mean ($n\sqrt{(a.b.c. \ldots)}$) of the reciprocal titre for each group at the times of vaccination and of challenge. In each case the mean for the 'homologous' serum-virus system reveals a satisfactory level of antibody immediately before challenge, whereas in the 'heterologous' system the antibody level was much lower at this time. Because the pre-vaccination mean antibody titres are not similar for all the groups, the logarithmic index of neutralization has been calculated (Table 3, columns 5 and 8). These indices demonstrate that, 3 weeks after vaccination, irrespective of the antigen used,

Antigen strain 13/61			Challenge strain 13/61				
			Serum titre				
Vaccine			Day 0		Day 22		
No.	Dilution	Animal No.	RV 11	13/61	RV 11	13/61	Lesions
614	0	DN 6	1/3	1/16	1/22	1/178	
		7	1/11	1/16	1/64	1/256	
		8	1/3	1/3	1/22	1/90	
		9	1/3	1/8	1/32	1/256	
		10	1/3	1/11	1/45	1/178	
		11	1/3	1/6	1/8	1/90	
		12	1/3	1/3	1/45	1/512	
		13	1/22	1/3	1/90	1/512	
		14	1/22	1/45	1/64	1/512	
		15	1/3	1/3	1/32	1/64	
615	0	DM 86	1/3	1/8	1/16	1/128	
		87	1/3	1/22	1/11	1/128	
		88	1/6	1/3	1/11	1/90	
		89	1/22	1/22	1/11	1/64	
		90	1/11	1/3	1/8	1/90	
		91	1/11	1/16	1/11	1/128	
		92	1/8	1/45	1/3	1/90	
		93	1/8	1/11	1/8	1/64	
		94	1/16	1/22	1/16	1/90	
		95	1/22	1/45	1/22	1/256	
615	1/3	DM 96	1/8	1/11	1/32	1/128	
		97	1/3	1/22	N.A.	1/45	
		98	1/3	1/3	1/6	1/45	
		99	1/3	1/16	1/3	1/64	
		DN 0	1/3	1/11	1/22	1/64	
		1	1/3	1/8	1/45	1/90	
		2	1/8	1/16	1/64	1/64	
		3	1/3	1/3	1/22	1/22	
		4	1/3	1/3	1/16	1/64	
		5	1/3	1/16	1/11	1/178	
Unvaccinated controls		DN 41					
		42					
		43					
		44					
		DM 80					
		81					
		82					
		83					
		84					
		85					



Antigen strain 13/61			Challenge strain RV 11				
			Serum titre				
Vaccine		Animal	Day 0		Day 22		
No.	Dilution	No.	RV 11	13/61	RV 11	13/61	Lesions
614	0	DN 65	1/3	1/8	1/22	1/178	
		66	1/3	1/3	1/11	1/178	
		67	N.A.	N.A.	1/22	1/90	
		68	1/3	1/3	1/22	1/90	
		69	1/3	1/11	1/3	1/64	
		70	1/3	1/3	1/3	1/90	
		71	1/3	1/11	1/3	1/32	
		72	1/3	1/3	1/22	1/90	
		73	1/3	1/3	1/22	1/90	
		74	1/3	1/3	1/8	1/64	
615	0	DN 45	1/8	1/3	1/22	1/90	
		46	1/11	1/22	1/32	1/90	
		47	1/6	1/11	1/3	1/32	
		48	1/3	1/3	1/3	1/90	
		49	1/8	1/3	1/22	1/90	
		50	1/3	1/3	1/8	1/22	
		51	1/4	1/8	1/16	1/64	
		52	1/3	1/3	1/8	1/45	
		53	1/8	1/8	1/45	1/90	
		54	1/8	1/16	1/16	1/11	
615	1/3	DN 55	1/3	1/8	1/22	1/16	
		56	1/3	1/3	1/22	1/3	
		57	1/11	1/8	1/11	1/90	
		58	1/8	1/11	1/8	1/45	
		59	1/3	1/3	1/11	1/22	
		60	1/11	1/22	1/11	1/45	
		61	1/3	1/4	1/8	1/45	
		62	1/6	1/6	1/11	1/22	
		63	1/3	1/11	1/3	1/90	
		64	1/3	N.A.	1/3	1/90	
Unvaccinated controls		DO 1					
		2					
		3					
		4					

Fig. 2. Neutralizing antibody responses of cattle to SA 13/61 antigen and reactions to challenge after 22 days. N.A., Results not available.

Table 3. *Geometric means of the reciprocal neutralization titres of sera from vaccinated cattle tested against virus of strains RV* 11 *and SA* 13/61

		Mean reciprocal neutralization titre against virus strains					
		RV 11			SA 13/61		
Experiment no.	Antigen	day 0 (*a*)	day 22 (*b*)	N.I.* log *b*–log *a*	day 0 (*c*)	day 22 (*d*)	N.I.* log *d*–log *c*
1	RV 11	7·2	93·3	1·11	11·7	14·3	0·09
2	RV 11	8·9	96·3	1·04	5·3	25·4	0·68
3	SA 13/61	6·7	19·2	0·46	10·1	146·4	1·16
4	SA 13/61	4·1	11·3	0·44	5·2	66·9	1·11

* Neutralization index.

the mean serum-neutralization titre has increased approximately threefold for the 'heterologous' serum-virus system and approximately 12-fold for the 'homologous' system.

Despite the wide variations of individual response revealed by serum neutralization tests, Figs. 1 and 2 demonstrate a good correlation between the serum

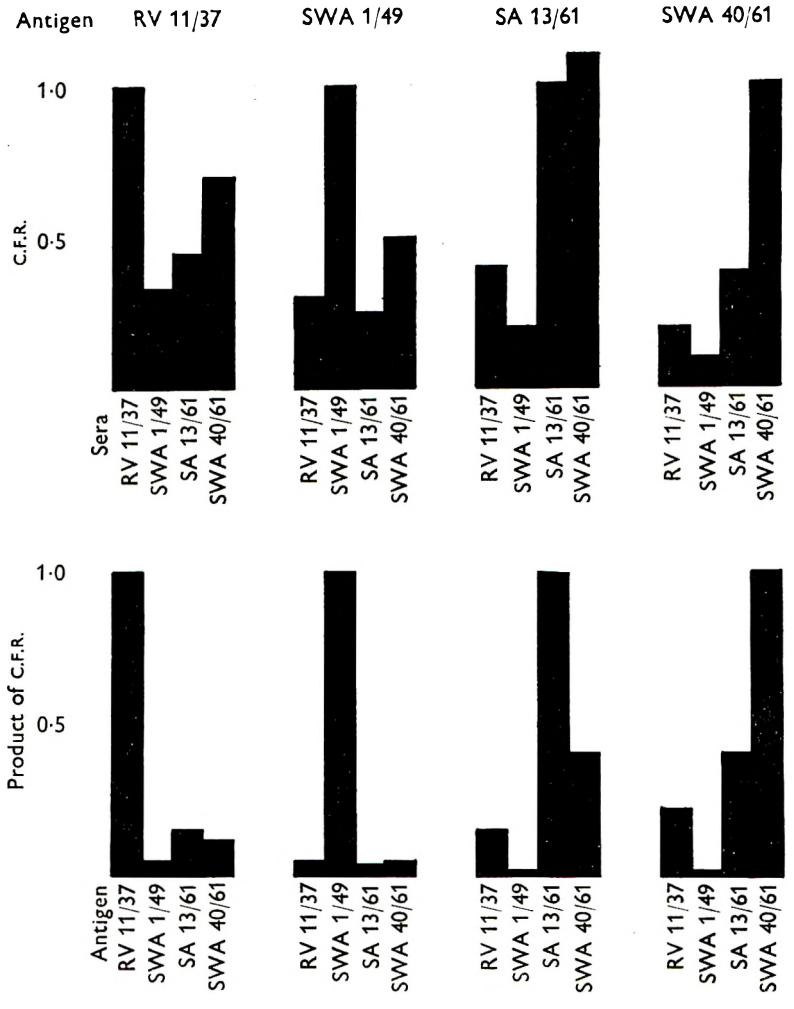


Fig. 3. Cross fixation ratios and their products for four strains of F.M.D. virus with strain-specific sera.

titre at the time of challenge and the clinical results of the challenge for each animal. Notwithstanding a few exceptions, it may be stated that cattle in which a titre of 1/45 is evoked are unlikely to develop a generalized infection as a result of the inoculation of 10^4 ID 50 of virus at each of 10 sites on the tongue; generalization is very likely to occur, however, in cattle whose neutralization titres are 1/22 or less.

4. *Complement-fixation tests*

During a survey of virus strains of type SAT 1, C.F. tests revealed (Davie, 1962) that the strains investigated could be classified into at least six distinct subtype groups. The strains RV 11, SA 13/61 and the stock type-specific strain SWA 1/49,

used for routine diagnostic tests in the World Reference Laboratory, each represent a separate subtype group.

The relationships of these strains and of strain SWA 40/61, isolated from the recent outbreak in South-West Africa, are illustrated by the histograms in Fig. 3. Because the antiserum of strain SWA 40/61 is of very high titre and the strain possesses some antigenic similarity to strain SA 13/61, its C.F.R. with strain SA 13/61 antigen is greater than unity. The cross-fixation product of the two antigens is 0·42.

The C.F.R. of strains RV 11 and SA 13/61 are small and the cross-fixation product is 0·17. This evidence of a wide antigenic difference between the strains is confirmed by the other tests described in this paper.

DISCUSSION

Although it seems at present that most strains of the virus of F.M.D. can be grouped into distinct types by serological and cross immunity tests, there is an increasing volume of evidence of wide variation within these type groups. Doubt may even exist if two strains at the extremes of the spectrum of variation within a type can be classified as of the same type. When, however, two such strains are related serologically to the intermediate strains within the type, and when tests reveal some cross immunity, albeit not complete, it would appear best to continue to refer to the strains as of the same type. Evolutionary progress may result in even greater subtype variation and so may make type definition even more difficult.

In the work reported here, the two strains concerned failed to give complete cross immunity in tests in guinea-pigs; and this may be considered to indicate an unusually great degree of dissimilarity between the strains. Shortage of cattle accommodation has precluded further examination, by cross-protection tests, of the antigenic relationship between these strains and other strains of type SAT 1, but the results of vaccination trials in the field would suggest that the classification of both strains as type SAT 1 is justified.

The complete protection of cattle evoked by vaccination with two strains of type SAT 1 against a severe experimental infection with the homologous strain is amply demonstrated in the foregoing experiments. This immunity contrasts remarkably with the almost complete lack of resistance to virus of the heterologous strain, although the animals in these groups all possessed serum antibody titres against virus of the vaccine strain which might have been expected to protect them (Figs. 1 and 2) against the homologous strain. The serum antibody titres against the heterologous strain were uniformly low and were consistent with the clinical results. Whether such low mean neutralizing antibody levels would be of any effect against *natural* exposure, which might be less severe than our experimental intradermal inoculation, remains a matter for speculation. It is unlikely, however, that the protection resulting from these liminal values would be of very long duration unless it was quickly reinforced by 'field virus'.

Figure 4 illustrates (diagrammatically only) the probable effect of strain differences on the performance of vaccines in the field; the regression curve for

this diagram is derived from data relating to a similar vaccine of type ASIA 1. If natural exposure occurred during the period represented by the shaded area of the graph, herd resistance might be reinforced without the appearance of clinical disease and thus a strain of considerable antigenic dissimilarity from the field strain would appear to evoke satisfactory immunity. Outside this period only the homologous strain, or a strain very similar to it, would produce satisfactory results.

The results of the use of vaccines under field conditions may be modified considerably by the interval between vaccination and exposure and by a variety of other extraneous factors which may mask or exacerbate the effects of antigenic differences between the vaccine strain and the wild strain of virus. Nevertheless, if C.F. tests demonstrate that antigenic dissimilarity is not very great, it appears that vaccination may provide some protection against natural exposure to a fully

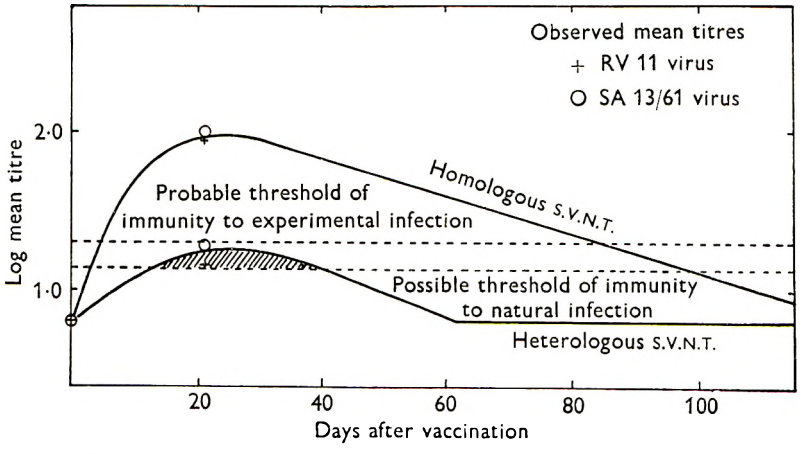


Fig. 4. Diagram illustrating the effect of strain differences on the resistance of cattle to experimental and natural infection.

invasive field strain of a somewhat different antigenic constitution. Thus, preliminary reports indicate that an inactivated vaccine of strain SA 13/61, which is being used currently against the outbreak of F.M.D. from which strain SWA 40/61 was isolated, has given generally satisfactory results (about 95 % protection) under African conditions in which exposure to infection probably occurred fairly soon after vaccination. The relatively close antigenic relationship between the two strains is shown in Fig. 3. These field trials have been the subject of a preliminary report (Galloway, 1962). Figure 3 also illustrates the relationship between these strains, strain RV 11, and the standard SAT 1 diagnostic strain SWA 1/49. Although the use for vaccines of strains which, though not identical, are shown to be antigenically related closely may be dictated by practical considerations, the controlled experiments suggest that *optimum* results from vaccination may be expected only when the homologous strain is incorporated in the vaccine.

As a consequence of experience gained with a pilot-scale plant at this Institute, little difficulty has been encountered in culturing F.M.D. virus of many strains to a high titre (10^7—$10^{8.5}$ ID50/ml.). Furthermore, it has been possible to produce virus of some strains on a 500 l. batch scale within 2 weeks of primary culture.

This ability to produce quickly large quantities of strain-specific vaccine suggests that, in the parts of the world where vaccination is acceptable as a control measure, future vaccination campaigns might commence with vaccine from existing stocks of the strain shown by C.F. tests to resemble the field strain most closely, but, if a substantial difference existed between stock and field strain, as soon as possible thereafter the use of this vaccine would be superseded by vaccine prepared from the local strain of virus of adequate antigenicity.

SUMMARY

Antigenic differences between the strains RV 11 and SA 13/61 of foot-and-mouth disease virus (type SAT 1) were studied *in vivo* by cross-protection tests. Cattle inoculated with formolized antigen of either strain developed good immunity to experimental infection with the identical strain but little resistance to the other strain.

In vitro the results of complement fixation tests and of serum-virus neutralization tests in tissue culture were consistent with the observations made *in vivo*. The results of studies on the serological relationships between four strains of type SAT 1 are presented.

The importance of strain differences in the epizootiology and control of the disease is discussed briefly.

The authors wish to thank Dr I. A. Galloway and Dr J. B. Brooksby for their advice and criticism, and to acknowledge the valuable technical assistance of Mr K. Herniman, Mr R. L. G. King and Mr E. Scoates.

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Variations in the clinical pattern of experimentally induced colds

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Studies of colds experimentally induced in volunteers have been in progress at the Common Cold Research Unit* of the National Institute for Medical Research for many years. This paper is based on an analysis of the results of the first 253 trials, which took place from August 1946 to July 1957.

Conduct of the trials

The organization of the work of the Unit has been previously described (Common Cold Research Unit, 1947; Andrewes, 1949; Lovelock, Porterfield, Roden, Somerville & Andrewes, 1952). Each trial began with the intake of a number of volunteers who, soon after their arrival at the Unit, were segregated, usually in pairs, for 9 days. They were observed for a few days and medically examined in order to determine their fitness to participate in the subsequent experimental procedures. Any volunteer who showed signs or symptoms suggestive of the common cold during this preliminary period of observation was excluded from the trial, and so was any companion sharing the same living quarters at the Unit. Volunteers were also excluded if they were found to be unsuitable, either on general grounds or by reason of any acute infection, abnormal quantity of nasal discharge or evidence of allergy.

The experimental procedures adopted in any trial were chosen in advance and the numbers of volunteers to be allocated to each procedure were decided at the end of the preliminary period of observation. For the purposes of allocation each segregated group of volunteers was treated as a single unit. The allocation was made by means of random sampling numbers. Neither the volunteers nor the clinical observers were aware of the nature of the experimental materials used until the final clinical assessments had been recorded.

The general conduct of the trials was the same throughout the years under review, though some modifications of detail were introduced from time to time. For example, the upper age limit for volunteers, which was originally 40 years, was raised in 1951 to 45 years. The lower age limit of 18 years was not altered. Until 1952 the preliminary period of observation was usually 3 days, though occasionally a 4-day period was chosen in order to attempt a higher degree of precision by reducing the possibility of the late development of natural infections after the experimental procedures had begun. Subsequently, 4 days became the general routine, except for work on experimentally induced colds of relatively long

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incubation, when a preliminary period of 2 days was adopted. The length of time for which volunteers remained under observation, after the experimental procedures had begun, varied, therefore, from 5 to 7 days.

Every effort was directed to the maintenance of continuity and uniformity of the records. Observations were entered daily on each volunteer's medical examination chart, which listed the main symptoms and signs on which the clinical assessments were based. Mouth temperatures were taken twice daily. Most of the observations and assessments were made by one of four medically qualified men—D. K. M. Chalmers (1946–47), T. Sommerville (1947–51), A. T. Roden (1951–56) and J. W. Field (1956–57). Dr C. H. Andrewes, who was in charge of the programme of scientific work throughout the period, helped to preserve uniformity.

Clinical assessments

Three main categories of assessment were adopted: (i) no cold, (ii) doubtful or abortive cold, and (iii) definite cold, the last being graded as mild, moderate or severe. A system of scoring points for diagnostic symptoms and signs was practised, but no arbitrary levels for the total score were fixed and the final assessment was essentially a clinical judgement.

Analysis of the records

A scheme for the analysis of the records was drawn up in 1955, in consultation with Dr E. R. Bransby, of the Ministry of Health, and Mr S. F. King, of the Treasury. Coded sheets of information suitable for transference to Powers-Samas punch-cards were prepared at the Unit. The punching and sorting of the cards was kindly undertaken by the Statistics Department of the Ministry of Labour and National Service.

A separate punch-card was prepared from the record of each occasion on which a volunteer was admitted to the Unit. It carried the following information:

Identification numbers of the volunteer, the trial and the living quarters occupied.
Number of persons sharing the living quarters.
Number of occasions on which the volunteer had been admitted to the Unit.
Month in which admitted.
Sex of volunteer and of companions, if any.
Age.
History of past frequency of colds.
Length of time since most recent cold.
History of tonsillectomy.
Duration of preliminary observation.
Duration of segregation.
Reason, if any, for exclusion from the trial.
Final clinical assessment.
Incubation period of cold, if any.
Symptoms persisting or developing after discharge from the Unit.
Nature of the experimental procedure.
Clinical observations.

The results given in this paper have been derived partly from a general analysis of the records and partly from a more detailed analysis of the clinical effects of different strains of virus.

Experimental procedures

For the purposes of a general analysis the experimental procedures were classified into three groups: (i) positive control, (ii) negative control, and (iii) other experimental procedures.

The positive control procedures consisted of the administration by nasal instillation of material, presumed to contain common cold virus, from the nasal washings of persons suffering from colds, either naturally acquired or experimentally induced. The negative control procedures consisted of the administration by nasal instillation of some presumably inert fluid. The other experimental procedures comprised the administration of materials under test for the presence of virus and various other forms of exposure to possible risk of infection.

Nasal washings for the positive control procedures were prepared, either by centrifugation, or by filtration through a collodion membrane of average pore diameter 0·65–0·7 μ, and stored at $-76°$ C. The quantity of material available from a single washing was limited and, although such material was often used, it seldom sufficed for administration to more than a total of twenty volunteers. Pooled washings, collected either from several persons or from the same person on more than one occasion, provided larger quantities of material, enough for administration to fifty volunteers or more.

The materials used for the negative control procedures comprised sterile normal saline, sterile broth saline and uninoculated egg or tissue culture fluids.

A wide variety of materials and methods was used for the other experimental procedures and it is not proposed to examine these in detail in this paper.

Strains of virus

During the period under review six strains of virus were propagated by serial transfer from volunteer to volunteer, using either individual or pooled nasal washings. The pedigrees of these strains were as follows:

Strain H.W. was present in a pool of nasal washings collected during an outbreak of minor upper respiratory tract infection at an English public school. This strain was propagated to the eighth serial transfer from the original material without apparent alteration in its pathogenic effects.

Strain C.W. was derived from a volunteer who developed a natural infection in one of the trials during the preliminary period of observation.

Strain R.D. was obtained from a volunteer who developed a cold after the administration of egg culture material.

Strains J.P. and D.C. each originated from a natural infection of a member of the staff of the Unit. The D.C. strain was subsequently propagated in tissue culture (Andrewes, Chaproniere, Gompels, Pereira & Roden, 1953; Tyrrell, Bynoe, Buckland & Hayflick, 1962) as well as by human passage.

Strain G.E. had an unusual history in that it was obtained from a volunteer who

developed a typical common cold after the administration of a culture of Type 1 adenovirus. The strain was propagated by human passage to the third serial transfer and no adenovirus was recovered in nasal washings either from the original volunteer or from others subsequently infected.

In addition to these six pedigree strains various other strains from miscellaneous sources were tested from time to time.

Clinical observations

For the purposes of a detailed analysis the clinical observations, which had been entered daily on the volunteers' medical examination charts, were classified retrospectively according to a uniform plan. Six signs and symptoms—pyrexia, coryza, purulent nasal discharge, nasal obstruction, sore throat and cough—were selected for study and each was graded as absent, slight and transient, slight and persistent, marked and transient or marked and persistent.

A symptom or sign was graded as transient if it was recorded as present during the experimental period of observation on one day only and as persistent if it was recorded as present on more than one day. Mouth temperatures of 98·8° to 99·8° F. were graded as slight pyrexia, and of 100° F. or more as marked pyrexia. Increased nasal discharge up to a maximum of eight paper handkerchiefs (or one fully soiled linen handkerchief) per day was graded as slight coryza and beyond this maximum as marked coryza. The remaining signs and symptoms had already been graded as slight or marked on the medical examination charts. The presence of purulent nasal discharge or of nasal obstruction was an objective finding on the part of the clinical observer. The grading of sore throat depended on the subjective observations of the volunteer and the grading of cough was based partly on subjective and partly on objective findings.

This retrospective classification of symptoms and signs in no way affected the final clinical assessments or the incubation periods already recorded.

RESULTS

In the first 253 trials, 3803 individuals were admitted to the Unit on a total of 5290 occasions, an average of twenty-one volunteers in attendance at each trial. The total number of exclusions was 855, of which 138 were on general medical grounds, 289 for signs or symptoms suggestive of the common cold, 137 by reason of an abnormal quantity of nasal discharge, fifteen on account of acute infections other than the common cold, 220 in contact with common cold or other infection and fifty-six for personal or other reasons.

The total number of volunteers who were included in the trials, counting each re-admission as a new entry, was 4435. Sixty-two of these were excluded from the analysis of the experimental results, either because the preliminary period of observation was less than 2 days or because the period of segregation was less than 9 days. This left a total of 4373, of whom 993 were allocated to positive control, 526 to negative control and 2854 to other experimental procedures.

The proportion of men among the volunteers allocated to each of these procedures was:

Positive control	447/993 (45·0 %)
Negative control	220/526 (41·8 %)
Other experimental	1253/2854 (43·9 %)

The respective proportions of volunteers less than 30 years old were:

Positive control	640/993 (64·5 %)
Negative control	331/526 (62·9 %)
Other experimental	1770/2854 (62·0 %)

The observed differences, in each instance less than twice the corresponding standard error, indicated that the groups were reasonably similar in sex and age composition. There were, however, marked disparities in the seasonal allocations. The proportions for months of admission October to March were:

Positive control	457/993 (46·0 %)
Negative control	166/526 (31·6 %)
Other experimental	1173/2854 (41·1 %)

The observed differences, all more than twice, and as regards the negative control group more than three times, the corresponding standard errors, revealed a bias in favour of positive control and against negative control procedures during the winter months.

Table 1. *Final clinical assessments in relation to experimental procedures*

	Experimental group							
	Positive control		Negative control		Other		Total	
Final clinical assessment	No. of volunteers	Percentage of group	No. of volunteers	Percentage of group	No. of volunteers	Percentage of group	No. of volunteers	Percentage of total
No cold or other illness	514	51·8	474	90·1	2307	80·8	3295	75·4
Doubtful or abortive cold	89	9·0	34	6·5	194	6·8	317	7·2
Definite cold: Mild	206	20·7	15	2·8	217	7·6	438	10·0
Definite cold: Moderate	159	16·0	3	0·6	91	3·2	253	5·8
Definite cold: Severe	21	2·1	—	—	13	0·5	34	0·8
Other forms of illness	4	0·4	—	—	32	1·1	36	0·8
Total	993	100·0	526	100·0	2854	100·0	4373	100·0

Table 1 shows the final clinical assessments of the 4373 volunteers included in the analysis of the experimental results. In the positive control group 386/993 (38·9 %) developed definite colds, compared with 18/526 (3·4 %) in the negative control group, a most significant difference statistically. The disparity between the two groups increased with the severity of the colds. The incidence of doubtful or abortive colds, 89/993 (9·0 %) and 34/526 (6·5 %) respectively, was not grossly dissimilar. Although the difference is less than twice its standard error, a partition of χ^2, or a discriminant analysis, shows that it is statistically very highly significant ($P < 0{\cdot}001$).

The attack rates of definite colds in the positive control group were examined in relation to each of the following properties of the individual volunteers: sex, age, past frequency of colds, length of time since most recent cold, history of tonsillectomy, number of persons sharing the same living quarters and number of occasions on which the individual had been admitted to the Unit. No difference exceeding twice the standard error was detected.

Table 2. *Seasonal distribution of experimental colds (positive control group)*

	Month of admission				
	Jan.–Mar.	Apr.–June	July–Sept.	Oct.–Dec.	Total
No. of volunteers	324	256	280	133	993
No. of colds: Definite	111	107	122	46	386
No. of colds: Doubtful or abortive	23	28	21	17	89
Percentage of volunteers who developed colds	41·4	52·8	51·1	47·4	47·9

Table 3. *Seasonal distribution of natural colds (volunteers excluded from trials)*

	Month of admission				
	Jan.–Mar.	Apr.–June	July–Sept.	Oct.–Dec.	Total
No. of volunteers	1417	1436	1553	884	5290
No. of colds (definite or doubtful)	103	64	48	74	289
Percentage of volunteers who developed colds	7·3	4·5	3·1	8·4	5·5

The seasonal distribution of all colds, definite and doubtful, in the positive control group is shown in Table 2. For purposes of comparison Table 3 shows the seasonal distribution of volunteers excluded from trials by reason of signs or symptoms suggestive of the common cold. A substantial number of these volunteers left the Unit soon after the onset of their symptoms and it was not possible, therefore, to classify all the natural colds into definite or doubtful categories. The distribution of the natural colds conformed to the known seasonal incidence of the disease, whereas the attack rate of the experimental colds was distinctly higher during the months April to September than from October to March. The observed difference, which was more than twice the standard error, was accounted for mainly by a lower attack rate of experimental colds among males during the winter months.

The proportions of definite colds among males in the positive control group were 65/221 (29·4 %) from October to March, and 102/226 (45·1 %) from April to September, a difference more than three times the standard error. The corresponding attack rates among females were 92/236 (39·0 %) from October to March and 127/310 (41·0 %) from April to September, a difference which was not

statistically significant. The difference between male and female attack rates from October to March was more than twice the standard error, but there was no statistically significant difference between the respective attack rates from April to September, or for the year as a whole.

Allocation of the virus strains

The different strains of virus, which were used for the positive control procedures, were administered to volunteers over varying periods of time: H.W., 1946–52; C.W., 1947–48; R.D., 1950; J.P., 1951–52; D.C., 1953–56; G.E., 1954–55; miscellaneous, 1946–57. The numbers of volunteers were, respectively: H.W., 282; C.W., 57; R.D., 49; J.P., 114; D.C., 139; G.E., 69; miscellaneous, 283.

There were minor differences as regards sex composition, the proportions of men being:

H.W.	133/282 (47·2 %)	J.P.	61/114 (53·5 %)
C.W.	25/57 (43·9 %)	D.C.	51/139 (36·7 %)
R.D.	20/49 (40·8 %)	G.E.	25/69 (36·2 %)

Miscellaneous 132/283 (46·6 %)

($\chi^2 = 10{\cdot}58$, $n = 6$, $0{\cdot}2 > P > 0{\cdot}1$)

The age composition showed significant disparities between some of the groups, which were attributable to a tendency for the average age of volunteers to rise as the work of the Unit progressed. The proportions of volunteers less than 30 years old were:

H.W.	203/282 (72·0 %)	J.P.	68/114 (59·7 %)
C.W.	42/57 (73·7 %)	D.C.	79/139 (56·8 %)
R.D.	29/49 (59·2 %)	G.E.	35/69 (50·7 %)

Miscellaneous 184/283 (65·0 %)

($\chi^2 = 20{\cdot}15$, $n = 6$, $P < 0{\cdot}01$)

Marked differences in the seasonal allocations were also evident. One strain (R.D.) was tested only during the months of May to September 1950. The remaining strains were tested both in winter and in summer. The allocations for months of admission October to March were:

H.W.	152/282 (53·9 %)	D.C.	56/139 (40·3 %)
C.W.	34/57 (59·6 %)	G.E.	31/69 (44·9 %)
J.P.	30/114 (26·3 %)	Miscellaneous	154/283 (54·4 %)

($\chi^2 = 36{\cdot}70$, $n = 5$, $P < 0{\cdot}001$)

Incidence of doubtful colds

Table 4 shows the final clinical assessments in the positive control group in relation to the strains of virus used. The incidence of doubtful or abortive colds was uniformly less than that of definite colds and, with each strain of virus, the assessments tended to fall into the categories of either no cold or definite cold, with relatively few doubtful cases. Two of the strains (C.W. and G.E.) were associated with an unusually low proportion of doubtful colds, but the numbers involved were small and none of the entries in this row of Table 4 differs from

expectation by more than twice its standard error. Partitioning the total χ^2 shows no significant difference ($P > 0{\cdot}05$) between the virus strains in the proportion of doubtful or abortive colds they produced. Nevertheless, it was the clinical impression that the G.E. strain, at least, gave particularly clear-cut results, although most of the colds associated with it were graded as mild.

Although statistically significant, the meaning of the doubtful or abortive colds was considered to be obscure, since they occurred with considerable frequency in the negative control group (see Table 1). For this reason the comparisons of the virus strains which now follow ignore all assessments in the doubtful category.

Table 4. *Final clinical assessments in relation to strains of virus*

	Strain of virus							Total no. of volunteers
	H.W.	C.W.	R.D.	J.P.	D.C.	G.E.	Miscellaneous	
Final clinical assessment								
No cold or other illness	126	21	25	73	76	27	166	514
Doubtful or abortive cold	31	3	4	8	15	2	26	89
Definite cold: Mild	62	12	15	19	20	28	50	206
Definite cold: Moderate	57	20	5	11	22	11	33	159
Definite cold: Severe	6	1	—	2	6	—	6	21
Other forms of illness	—	—	—	1	—	1	2	4
Total	282	57	49	114	139	69	283	993

Table 5. *Clinical attack rates of experimentally induced colds*

	Strain of virus							
	H.W.	C.W.	R.D.	J.P.	D.C.	G.E.	Misc.	Total
Oct. to Mar.								
No. of volunteers	152	34	Nil	30	56	31	154	457
No. of definite colds	60	20	Nil	7	17	15	38	157
Percentage	39·5	58·8	Nil	23·3	30·4	48·4	24·7	34·4
Apr. to Sept.								
No. of volunteers	130	23	49	84	83	38	129	536
No. of definite colds	65	13	20	25	31	24	51	229
Percentage	50·0	56·5	40·8	29·8	37·3	63·2	39·5	42·7

Incidence of definite colds

The proportions of volunteers who developed definite colds were:

H.W. 125/282 (44·3 %) J.P. 32/114 (28·1 %)
C.W. 33/57 (57·9 %) D.C. 48/139 (34·5 %)
R.D. 20/49 (40·8 %) G.E. 39/69 (56·5 %)
Miscellaneous 89/283 (31·4 %)

These differences were most unlikely to have arisen by chance ($\chi^2 = 35{\cdot}16$, $n = 6$, $P < 0{\cdot}001$).

Table 5 shows the number of colds associated with each of the virus strains during the months October to March and April to September, respectively. Five of the pedigree strains were tested at both these periods of the year. All but one

(C.W.) gave a higher clinical attack rate during the summer than during the winter and so did the miscellaneous strains. The differences between the attack rates associated with strains other than C.W. were of roughly the same order both in summer and in winter.

Severity of illness

The proportions of colds graded as moderate or severe were:

H.W.	63/125 (50·4 %)	J.P.	13/32 (40·7 %)
C.W.	21/33 (63·6 %)	D.C.	28/48 (58·3 %)
R.D.	5/20 (25·0 %)	G.E.	11/39 (28·2 %)

Miscellaneous 39/89 (43·8 %)

These differences were unlikely to have been fortuitous ($\chi^2 = 16{\cdot}95$, $n = 6$, $P < 0{\cdot}01$).

Some variability in the grading of the severity of illness was shown by different clinical observers, and by the same observer at different times, but the variations were not generally of a gross degree. Most of the assessments associated with the J.P., D.C. and G.E. strains were made by one observer.

Table 6. *Incubation periods of experimentally induced colds*

	Incubation periods (days)						Total no. of colds
	0–	1–	2–	3–	4–	5–	
Strain of virus							
H.W.	12	70	29	10	2	2	125
C.W.	5	10	12	3	2	1	33
R.D.	—	6	8	4	1	1	20
J.P.	6	18	4	1	3	—	32
D.C.	13	28	6	1	—	*	48
G.E.	2	2	14	11	7	3	39
Misc.	21	45	17	4	1	1	89
Total	59	179	90	34	16	8	386

* Volunteers usually no longer under observation (see 'Conduct of the trials').

Incubation period

Table 6 shows the incubation periods, grouped in 24 hr. intervals from 12 noon of the day of inoculation to the day of onset of illness, of the 386 colds which developed in the positive control group. The mean periods, in days, calculated from the central values of the 24 hr. intervals were: H.W., 1·91; C.W., 2·20; R.D., 2·65; J.P., 1·78; D.C., 1·40; G.E., 3·22; miscellaneous, 1·62. (Variance ratio 16·83, for degrees of freedom 6 and 379, $P < 0{\cdot}001$.) The proportions of colds with an incubation period of less than 2 days were: H.W., 82/125 (65·6 %); C.W., 15/33 (45·5 %); R.D., 6/20 (30·0 %); J.P., 24/32 (75·0 %); D.C., 41/48 (85·4 %); G.E., 4/39 (10·3 %); miscellaneous 66/89 (73·9 %).

The original data on the D.C. and G.E. strains, between which the greatest disparity of incubation period existed, were re-examined to determine whether differences in the rate of evolution of symptoms could have influenced the observer's judgement of the day of onset of illness. Two further sets of measure-

ments were compared; from the time of inoculation to (i) the development of the first marked symptom, (ii) the onset of persistent coryza; and in each instance the distinctive distributions of the incubation periods were maintained.

The 125 colds associated with the H.W. strain were examined for a possible seasonal influence, but none was found. The mean incubation periods were 1·73 days from October to March and 2·07 days from April to September. The proportions of colds with an incubation period of less than 2 days were 42/60 (70·0 %) and 40/65 (61·5 %), respectively. Each of these differences was less than twice the corresponding standard error.

Duration of colds

Some of the volunteers recovered completely from experimentally induced colds before their departure from the Unit. Symptoms which developed, recurred or persisted after departure were recorded by the volunteers themselves on follow-up cards, which they were asked to return to the Unit a fortnight later by post. Although the information so obtained was not always complete or accurate, it was found possible in most instances to form a rough estimate of the duration of colds. Table 7 summarizes the findings. It will be seen that the colds associated with the G.E. strain, of relatively long incubation period, tended to be of short duration, only three of thirty-nine being known to have persisted for more than 2 weeks from the time of onset. In contrast, the colds associated with the D.C. strain, of

Table 7. *Estimated duration of symptoms*

	Duration (days) from onset				Total no. of colds
	Less than 7	7 to 13	14 or more	Not known	
Strain of virus					
H.W.	35	25	31	34	125
C.W.	17	5	1	10	33
R.D.	4	8	1	7	20
J.P.	11	11	9	1	32
D.C.	7	16	24	1	48
G.E.	12	23	3	1	39
Misc.	24	21	22	22	89
Total	110	109	91	76	386

relatively short incubation period, tended to be prolonged, no fewer than twenty-four of forty-eight persisting for 2 weeks or more. The duration of colds associated with the J.P. strain occupied an intermediate position. Information on the remaining strains was less complete, but there was evidence to suggest that colds associated with the C.W. strain were usually of short duration.

Respiratory symptoms, which had previously cleared, were reported in five cases to have recurred after departure. Two of these were associated with miscellaneous strains and one each with H.W., C.W. and G.E.

Relative frequency of symptoms

The first symptom to appear was usually soreness of the upper respiratory passages, followed or accompanied by increased nasal exudate. The soreness was referred sometimes to the upper part of the naso-pharynx, sometimes to the region of the fauces and occasionally to a lower level. Precise location was not always possible and the term sore throat was used to cover this symptom in a general way. Inspection of the throat was not particularly helpful. There was often marked reddening without soreness or other evidence of a cold, while a normal appearance was frequently maintained in the presence of obvious evidence of infection.

The most characteristic sign was coryza, or increased nasal discharge, the amount of which could be measured roughly by examining and counting the used handkerchiefs. Some degree of coryza was observed in almost every case assessed as a definite cold. In only five of the 386 colds under consideration was the diagnosis made in the absence of coryza. The exudate usually consisted at first of clear mucus and, in some cases, remained clear throughout the course of the illness. Muco-purulent discharge often developed at a later stage and, occasionally, was present at the time of onset of coryza. A feeling of nasal stuffiness was common and some degree of obstruction frequently detected. Cough was relatively less common and, when it occurred, tended to be a late manifestation.

The grading of severity was largely influenced by the presence of symptoms other than coryza and by the degree of malaise which accompanied them. Malaise, headache or sneezing, all fairly common, were found to have no significance when they occurred as isolated symptoms, and, although their presence might influence the grading of severity, contributed little to the diagnosis. Some signs, such as hoarseness and watering of the eyes, were highly characteristic, but relatively infrequent and invariably accompanied by other evidence of infection.

Slight pyrexia, usually transient, was sometimes observed, either at the onset or at the peak of illness, but many of the colds were completely afebrile. Mouth temperatures exceeding 99·8° F. were recorded in only nine of the 993 volunteers in the positive control group, eight of whom were among the 386 who developed colds.

Comparison of the virus strains, based on the presence or absence of individual symptoms or combinations of symptoms, revealed differences which became more distinct when those graded as slight had been excluded. Table 8 shows an analysis of the 386 colds according to the relative frequency of marked degrees of pyrexia, coryza, purulent nasal discharge, nasal obstruction, sore throat and cough. A partition of the 3-way χ^2, by Lancaster's method (Lancaster, 1951), showed not only that different strains varied in the overall frequency of symptoms they caused, but also that they were very definitely associated with certain symptoms, the second-order interaction χ^2 being highly significant, $P < 0{\cdot}001$.

Colds associated with the H.W. strain, which accounted for nearly one-third of the total, showed a frequency near the average for marked coryza and above the average for other respiratory symptoms. Strain D.C. resembled the H.W. strain

in the frequency of coryza, purulent nasal discharge and cough, but nasal obstruction and sore throat were well below the average. The miscellaneous strains also gave a frequency near the average for coryza, but other respiratory symptoms were either near or below the average.

Table 8. *Relative frequency of marked symptoms*

	No. of volunteers in whom marked symptoms occurred						
	Pyrexia	Coryza	Purulent nasal discharge	Nasal obstruction	Sore throat	Cough	Total no. of colds
Strain of virus							
H.W.	3 (2·4 %)	87 (69·6 %)	76 (60·8 %)	105 (84·0 %)	75 (60·0 %)	46 (36·8 %)	125
C.W.	—	27 (81·8 %)	15 (45·4 %)	31 (93·9 %)	13 (39·4 %)	5 (15·2 %)	33
R.D.	1 (5·0 %)	16 (80·0 %)	14 (70·0 %)	18 (90·0 %)	13 (65·0 %)	2 (10·0 %)	20
J.P.	2 (6·2 %)	13 (40·6 %)	14 (43·7 %)	18 (56·2 %)	23 (71·9 %)	12 (37·5 %)	32
D.C.	—	31 (64·5 %)	28 (58·3 %)	31 (64·5 %)	19 (39·6 %)	19 (39·6 %)	48
G.E.	—	31 (79·5 %)	6 (15·4 %)	31 (79·5 %)	9 (23·1 %)	4 (10·3 %)	39
Misc.	2 (2·2 %)	60 (67·4 %)	45 (50·5 %)	53 (59·5 %)	43 (48·3 %)	20 (22·5 %)	89
Total	8 (2·1 %)	265 (68·6 %)	198 (51·3 %)	287 (74·3 %)	195 (50·5 %)	108 (28·0 %)	386

Colds induced by the C.W. strain showed the highest frequencies for marked coryza and nasal obstruction, but other respiratory symptoms were below the average. The R.D. strain gave figures almost as high for coryza and nasal obstruction and well above the average for purulent nasal discharge and sore throat, but cough was infrequent. The G.E. strain, also, showed high figures for coryza and nasal obstruction, with exceptionally low frequencies of purulent nasal discharge, sore throat and cough. The comparative rarity of marked cough was a feature of all three of these eminently coryzal strains.

Colds associated with the J.P. strain were peculiar in the relative infrequency of marked nasal symptoms, with cough approximating to the proportions shown by the H.W. and D.C. strains and an exceptionally high frequency of marked sore throat.

Similar differences in the pattern of the clinical effects associated with the various strains were maintained when the records of the volunteers were examined for symptoms, irrespective of the final assessments. The differences appeared to be independent of season. Table 9 shows the number of volunteers who developed marked symptoms after administration of the H.W. strain during the months October to March and April to September respectively. The incidence of all symptoms was higher in the summer than in the winter, but their relative pro-

portions were much the same. The clinical patterns of illness associated with the other pedigree strains were also, as far as could be judged from rather small numbers, not materially affected by season.

Table 9. *Incidence of marked symptoms—H.W. strain*

	No. of volunteers in whom marked symptoms occurred						
	Pyrexia	Coryza	Purulent nasal discharge	Nasal obstruction	Sore throat	Cough	Total no. of volunteers
Oct. to Mar.	2	42	38	64	47	23	152
Apr. to Sept.	1	45	39	69	54	27	130
Total	3	87	77	133	101	50	282

DISCUSSION

The trials were designed with the primary object of testing experimental materials for the presence of common cold virus and, in this respect, were admirably suited to their purpose. The reliability of the observations and the validity of the assessments based on them have been discussed elsewhere (Roden, 1958). The bias which was detected towards the adoption of positive control procedures during the winter months did not invalidate a comparison between the positive and the negative control groups, partly because there was a gross difference of more than tenfold in the respective attack rates of definite colds and partly because it was found that the attack rate in the positive control group was in fact higher during the summer.

The heterogeneous nature of the experimental materials precluded any firm deductions concerning factors likely to have influenced the susceptibility of volunteers to infection. No laboratory method for the detection or titration of common cold virus was available at this time and there was no way either of verifying the identity of the strains propagated by human passage or of estimating the quantity of virus administered. If it were assumed, for the purpose of a general analysis, that equivalent doses of identical viruses were administered to all volunteers in the positive control group, the only factor which appeared to have influenced the number of experimentally induced colds was a seasonal one, associated with a lower clinical attack rate among males during the winter months. The seasonal variation could not be attributed to differences between the strains of virus used, though this was probably the explanation of the varying attack rates which were observed from year to year (Andrewes *et al.* 1953). A seasonal influence on susceptibility was not demonstrated in volunteer studies in the United States (Dowling, Jackson, Spiesman & Inouye, 1958) or by earlier analyses of data from the Common Cold Research Unit. The negative findings as regards the other factors examined must be accepted with reserve, since they may have reflected an experimental design inadequate to reveal differences which could have existed.

The diversity in attack rate, severity, incubation period, duration and clinical pattern of colds associated with different strains of virus was too great to be

attributed merely to errors of sampling. Variations in the dose of virus could be held to have explained the differences in the attack rates, but not the paradoxical finding that those strains (C.W. and G.E.) which gave the highest attack rates were associated with long incubation periods, short durations and low proportions of symptoms other than coryza and nasal obstruction. The season of the year was found to have little or no influence, either on the clinical pattern of colds associated with a particular strain or on the attack rate relative to that of other strains. Disparity in the age and sex of the volunteers was an inadequate explanation of the observed variations in clinical pattern, since strains H.W. and C.W., which differed considerably in their effects, were tested in groups of reasonably similar age and sex composition and so were strains D.C. and G.E. It is possible that some of the longer incubation periods arose from failure to infect volunteers by the experimental procedure, with subsequent cross-infection from a companion, but this could not have accounted for the widely different proportions of colds which developed within less than two days of inoculation. The conclusion was that the findings were explicable only in terms of qualitative differences between the strains. This is consistent with more recent work at the Unit where a number of common cold viruses have been grown in the laboratory, some of which differ in their cultural requirements (Tyrrell, Bynoe, Hitchcock, Pereira, Andrewes & Parsons, 1960) and clinical effects (Tyrrell & Bynoe, 1961). In the absence of laboratory tests there was no assurance that propagation of the same strain was taking place by human passage. The possibilities of coincidental natural infection or of activation of a latent virus must also be considered. On the whole the accidental propagation or activation of heterogeneous viruses would have tended to obscure the observed variations and, for this reason, the occurrence of either of these contingencies would have been unlikely to invalidate the general conclusion that there were differences between the strains under study.

To account for the seasonal variation in the attack rate of colds associated with the miscellaneous strains and at least four of the pedigree strains it is tempting to put forward the hypothesis that the resistance of volunteers to these strains was higher during the winter months as a consequence of recent exposure to natural infection. Reid, Williams & Hirch (1953), in an epidemiological investigation of colds among office workers, obtained evidence of a short-term immunity. A similar conclusion was reached by Lidwell & Williams (1961). Volunteer studies by Andrewes (1950), Jackson, Dowling & Anderson (1958) and Jackson & Dowling (1959) gave results which suggested that resistance to the common cold was of a specific character. Tyrrell & Bynoe (1961) have since shown that infection by cytopathic common cold viruses induces the production of specific neutralizing antibodies, which can be detected in the blood serum. As regards the present data, it is difficult to understand why an increased resistance to infection in the winter months should be restricted to the male sex. An alternative explanation for the findings may lie in the possibility that men who were more susceptible to the common cold tended not to come to the Unit during the winter and that those who did come tended to be excluded from the trials because of symptoms or signs of upper respiratory disease. Whatever the explanation may be there was clearly

some factor, associated with the nature of the volunteer groups, which differentiated the sexes as regards susceptibility to infection during the winter and ceased to operate during the summer; but it would be unjustifiable to infer that this necessarily applied to natural infections in the general community.

Jackson, Dowling, Spiesman & Boand (1958) described variations in the incubation period and duration of experimental colds, which were induced in volunteers by the instillation of nasal secretions collected from different donors, and in the time relationship of the average daily score of symptoms. In contrast with the results of the present analysis these investigators found that neither sex nor season nor the source of the infectious secretion was significantly associated with susceptibility to experimental infection and that there was a striking similarity in the illnesses caused by different secretions.

Future work on the common cold is likely to proceed in conjunction with laboratory tests for known serotypes of virus, which will bring greater precision to the findings. It is improbable, however, that anyone will be able to test specific strains of virus on large numbers of volunteers living in isolation. For this reason it is thought that the present analysis may provide useful background information, help to clear the path for subsequent investigations in this field, and draw attention to some of the difficulties which may arise in the interpretation of data derived from volunteer studies.

SUMMARY

An analysis has been made of the records of 5290 attendances at the Common Cold Research Unit, Salisbury, during the years 1946 to 1957. Materials prepared from the nasal washings of persons suffering from colds were administered to 993 volunteers, of whom 386 (38·9 %) showed definite evidence of infection within a few days. The attack rate of these experimentally induced colds was lower during the months October to March than from April to September. The difference was statistically significant as regards male but not as regards female volunteers.

Six strains of virus were propagated by passage from volunteer to volunteer, in one instance up to the eighth serial transfer from the original material, without apparent loss of pathogenic properties. Comparison of the clinical effects of these strains, and of a number of miscellaneous strains, revealed several differences which were minor in character but, statistically, highly significant. These comprised differences in attack rate, severity, incubation period, duration and relative frequency of symptoms, which could not be ascribed to seasonal or other influences.

It was concluded that the observed variations in the clinical pattern of the experimentally induced colds were attributable to qualitative differences in the strains of virus used.

I wish to thank Sir Christopher Andrewes, F.R.S., for his unfailing help and encouragement at every stage of this investigation and all my colleagues at the Common Cold Research Unit for much useful comment and discussion. I am indebted to the Statistics Department of the Ministry of Labour and National Service for processing the records and to Mr R. G. Carpenter for assistance with some of the statistical analyses.

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Naso-pharyngeal carriage of *Haemophilus influenzae* type B

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INTRODUCTION

Pittman (1931) showed that the bacterial species *Haemophilus influenzae* is divisible into S (capsulate) and R (non-capsulate) strains. She also distinguished among the capsulate strains six sero-types, *a*, *b*, *c*, *d*, *e* and *f* (1931 and later unpublished work).

Haemophilus influenzae is commonly present in the upper respiratory tracts of healthy children and adults (Blackburn *et al.* 1930; Straker, Hill & Lovell, 1939; Masters, Brumfitt, Mendez & Likar, 1958); and also in the sputum of patients with bronchial diseases (Mulder, 1940; May 1953, 1954; Allibone, Allison & Zinnemann, 1956). By routine use of appropriate media it can be found in many other situations (Rogers, Zinnemann & Foster, 1960). Most strains from all the sources mentioned so far are non-capsulate.

In contrast, the strains which cause meningitis, pneumonia, epiglottitis, suppurative arthritis and certain other acute infections are nearly always capsulate and of Pittman's type *b* (Pittman, 1931; Alexander, Ellis & Leidy, 1942; Thilenius & Carter, 1959).

The available information suggests that under normal conditions *H. influenzae* type *b* is carried in the upper respiratory tract by about 3 % of children under 5 years old and about 1 % of older children and adults (Dawson & Zinnemann, 1952; Masters *et al.* 1958). But Good, Fousek, Grossmann & Boisvert (1943) found carriers of such strains in the homes of 3 out of 5 children with illnesses due to *H. influenzae* type *b*; whereas they found none in the homes of 9 'control' children (3 carrying type *b* strains without associated symptoms and 6 from whom no such organisms had been grown).

Haemophilus influenzae was much the commonest cause of meningitis seen at the University College Hospital of the West Indies, Jamaica, in the years 1958–60 (Turk & Wynter, 1961). All the strains isolated belonged to type *b*. All the patients with haemophilus meningitis were children, most of them between the ages of 3 months and 2 years (cf. Smith, 1954; Ouyang & Ting, 1957; Karelitz, Desposito, Spinner & Isenberg, 1960). Partly because of this prominence of *H. influenzae* type *b* as a cause of meningitis in Jamaica, naso-pharyngeal carriage-rates for the whole species were studied in various samples of the island's population. The methods and findings of that survey have been reported in full elsewhere (Turk,

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1962). The present paper is primarily concerned with the frequencies of isolation of type *b* strains, and especially with the high concentrations found in some of the samples.

METHODS

Specimens

A single specimen was collected from each subject on any one occasion, by passing a fine, flexible wire swab along the floor of one nostril to the naso-pharynx. The swab was used within $2\frac{1}{2}$ hr. of collection to inoculate the two media described below.

Primary cultures

(*a*) Half of a horse-blood agar plate was inoculated and spread so as to give progressive thinning of the inoculum from the periphery to the half-way line. After the other half-plate had been similarly inoculated with another swab, *Staphylococcus aureus* was streaked across the plate at right angles to the half-way line, so as to pass through the middle of both inocula. After 24 hr. incubation the plates were examined for the presence of satellite colonies alongside the staphylococcal streaks.

(*b*) Each swab was also used to inoculate half a plate of a selective medium. This was made by adding 5 % of citrated horse blood to melted Veal Infusion Agar (Difco), heating the mixture in a boiling water-bath for 10 min., cooling to about 50 °C., and adding 1 % of supplement A (Difco) before pouring the plates. The supplement, as well as being an additional source of X- and V-factors, contained crystal violet, of which the final concentration was 1:714,000. This delayed the growth of many respiratory-tract organisms—notably staphylococci and neisseriae—while allowing *H. influenzae* to form colonies of up to 2 mm. in diameter after 24 hr. incubation at 37 °C.

Recognition of capsulate strains

Colonies on either of the primary media which resembled those of *H. influenzae* were subcultured to Levinthal's agar medium as modified by Alexander (1958). After 18 hr. incubation, the Levinthal's agar plates were examined by strong, obliquely transmitted light for the greenish iridescence of capsulate strains described by Pittman (1931). Capsulate strains were also distinguishable on this medium by their greater opacity, more mucoid surface appearance and tendency to confluence of colonies.

Typing of capsulate strains

All strains considered to be capsulate were typed by slide-agglutination, using sera bought from Burroughs, Wellcome and Co. in England; and by 'capsule-swelling' tests, using either sera made and kindly supplied by Miss Grace Leidy of the Presbyterian Hospital, New York, or those bought from Hyland Laboratories, Los Angeles. Sera from the two American sources usually gave convincing type-specific 'capsule-swelling' reactions with organisms from 18 hr. Levinthal's

agar cultures, though for a few strains it was necessary to use much younger cultures. The Burroughs, Wellcome sera did not give satisfactory 'capsule-swelling' reactions with over night cultures.

RESULTS

Non-Jamaican children

These were members of white families from Britain only temporarily resident in Jamaica. Specimens were collected from:

(*a*) 58 children, aged between 4 months and 13 years, swabbed within a few days of their arrival from Britain.

(*b*) 32 of the same children re-examined 7 months later.

(*c*) 37 other children, aged between 8 months and 11 years, who had lived in the island for periods exceeding 7 months.

Type *b* strains were recovered from one 3-year-old boy in (*a*) and from one 4-year-old and one 9-year-old girl in (*c*), giving an overall recovery rate of 2·4 per 100 swabs. The corresponding rate for all *H. influenzae* strains was 60·6 per 100 swabs.

Jamaican children giving 'normal' results

Nearly all of the children referred to under this and subsequent headings were at least partly of negro descent, most of them predominantly so; but a few pure Chinese children were included in some of the samples. Specimens were collected from:

(*a*) 100 babies, aged between 3 months and 2 years, attending a child welfare clinic.

(*b*) 200 school children, aged between 5 years and 9 years, representing 4 large schools with a total attendance of about 3000 pupils.

(*c*) 92 children, aged between 4 months and 14 years, living in 23 homes on a lower-income-group housing estate.

(*d*) 81 of these 92 children re-examined 4 months later, together with 5 other children from the same homes who had not been available on the first occasion.

Type *b* strains were recovered from 2 of the 100 babies in (*a*), from 7 of the 200 school children in (*b*), from 3 of the 92 children in (*c*) and from 2 of the 86 in (*d*), giving an overall recovery rate of 2·9 per 100 swabs. The highest concentration consisted of 4 strains found among the 60 representatives of one of the schools in (*b*).

The recovery rates for all *H. influenzae* strains were lower in each of these samples and in all age groups than those found among the non-Jamaican children, the overall figure being 40 per 100 swabs.

Families of meningitis patients

Swabs were obtained, in each case within 2 days of the patient's admission to hospital, from the families of 6 children with haemophilus meningitis. All available members of each family who lived in the same home as the patient were included—the 'home' being a house, a single room or a shack, according to the

economic status of the family. As shown in Table 1, *H. influenzae* type *b* was recovered from 5 of the 6 families, from 2 of the 8 parents and from 7 of the 14 siblings.

Table 1. *Families of meningitis patients*

Family number	Meningitis patient	Members of families: Found to carry *H. influenzae* type *b*	Members of families: Not found to carry H. *influenzae* type *b*
1	Male 2 years	F 4 years	F 5 months
		F 6 years	M 8 years F 10 years Mother
2	Female 3 years	—	M 1 year Mother Father
3	Male 5 months	F 2 years	F 5 years M 7 years Mother Father
4	Male 11 weeks	M 3 years Mother	F 4 years
5	Female 11 months	F 10 years	Mother
6	Female 6 months	F 2 years M 4 years Mother	—

M, male child (patient's brother or half-brother); F, female child (patient's sister).

An orphan home

(*a*) The senior house contained about 170 children, nearly all between the ages of 5 years and 15 years. Two visits were made at an interval of 4 months, and 329 swabs were collected. No type *b* strains were isolated. Recovery rates for all *H. influenzae* strains were 49·3 and 67·7 per 100 swabs on the two occasions.

(*b*) The junior house contained up to 50 children, aged between 1 year and 4 years, who had very little contact with those in the senior house. 4 visits were made, with the results shown in Table 2. Type *b* strains were considerably more common than in any of the preceding groups other than the families of meningitis patients. Recovery rates for all *H. influenzae* strains were also high.

(*c*) The nursery contained up to 15 babies not yet old enough to walk, and kept in separate cots in a room to which older children were not admitted. They were, however, cared for by the same staff as the children of the junior house, and several of them were promoted to the junior house during the period of observation. They gave such remarkable results when first examined that they were swabbed repeatedly. From the results shown in Table 3 it can be seen that:

(i) On each of the first 6 visits *H. influenzae* type *b* was isolated from between 70 and 38 % of the children present.

(ii) No child was proved to enter the nursery carrying a type *b* strain.

(iii) All the children who were in the nursery at the beginning of the period of observation carried such a strain at some time.

(iv) Only 3 of those admitted later failed to yield such a strain at some time, and 2 of these were only present during the latter part of the period.

(v) No child was observed to carry a type *b* strain for more than 3 months, though some left the nursery while still carrying such strains.

Table 2. *The orphan home junior house*

Date	Number of children	Number found to carry *H. influenzae* type *b*	Number found to carry *H. influenzae* all strains
17. viii. 60	49	5	33
31. viii. 60	49	5 (3)	36
27. ix. 60	48	8 (5)	39
28. xi. 60	46	6 (2)	39
Total	192	24	147

The figures in parentheses indicate the number of those found to carry type *b* strains who had also yielded such strains on previous occasions.

Table 3. *The orphan home nursery*

(August 1960 to March 1961)

Ref. no. of child	Age (months)	Sex	Date of admission	Results from swabs collected on 29 Aug.	31 Aug.	27 Sept.	12 Oct.	27 Oct.	28 Nov.	26 Jan.	2 Mar.
1	7	F	April	*b*	*b*	+	*b*	*b*	*b*+	.	.
2	12	F	April	*b*	+	(*b*)	.	.	(*b*)	.	.
3	7	M	April	*b*	*b*	*b*	*b*	*b*+	+	+	+
4	8	F	April	*b*	*b*	+	*b*	*b*+	*b*	.	.
5	4	F	May	*b*	*b*	*b*	*b*	.	.	.	.
6	9	M	June	–	–	+	*b*	.	(*b*)	.	.
7	2	F	June	+	+	*b*	*b*	*b*+	*b*	*b*	.
8	5	M	July	*b*	*b*	*b*	*b*	*b*	*b*	+	+
9	5	F	July	*b*	*b*	+	+	+	*b*	+	+
10	9	M	July	+	+	*b*+	+	+	+	*b*+	+
11	4	M	8 Sept.	.	.	–	–	–	*b*	*b*	–
12	3	F	9 Sept.	.	.	–	+	–	*b*+	.	.
13	?1	M	12 Sept.	.	.	–	–	+	+	+	+
14	4	M	14 Sept.	.	.	–	*b*	?	*b*	+	+
15	8	F	*	.	.	.	?	*b*+	*b*+	+	+
16	?8	M	*	.	.	.	–	–	+	*b*	*b*+
17	3	M	21 Nov.	.	.	.	.	.	–	+	*b*+
18	12	M	30 Nov.	.	.	.	.	.	.	+	+
19	5	F	24 Dec.	.	.	.	.	.	.	+	+

The age given for each child is that when first examined.

–, no *H. influenzae* grown; +, non-capsulate strains grown; *b*, type *b* grown; *b*+, type *b* and non-capsulate strains grown; (*b*) type *b* grown but the child was no longer in the nursery; ?, cultures overgrown by *Proteus* sp., with probable underlying *H. influenzae* which could not be obtained in pure culture; slide-agglutination and capsule-swelling tests for type *b* in the mixed growth negative; ., not present on this date; *, in the home before the period of observation began, but absent during August and early September.

(vi) At the last visit only two of the more recent arrivals yielded type *b* strains, whereas no such strains were isolated from 7 children then present who had carried them on previous occasions.

No case of meningitis or other illness attributable to *H. influenzae* occurred in the nursery immediately before, during or immediately after the period of these studies. Haemophilus meningitis had occurred there in the past, but the last case was in May 1959.

The study of this very interesting situation could not be continued because I left the island in April 1961.

A day-nursery and play-school

Children from low-income homes were left at this institution while their parents were at work. Its population therefore varied, but some children attended fairly regularly over long periods.

Table 4. *The day-nursery and play-school*

	October 1960						March 1961					
		Number found to carry *H. influenzae*						Number found to carry *H. influenzae*				
		Capsulate types						Capsulate types				
Grade	Number of children examined	*a*	*b*	*e*	*f*	All strains	Number of children examined	*a*	*b*	*e*	*f*	All strains
A	10	0	0	0	0	5	10	0	0	0	0	3
B	15	0	0	0	0	9	15	0	1	1	1	13
C	16	0	3	1	0	10	13	0	0	3	0	9
D	10	0	2	0	0	8	12	0	1	4	2	10
Play-school	34	1	4	0	0	18	0					
Total	85	1	9	1	0	50 (59%)	50	0	2	8	3	35 (70%)

The nursery children were graded according to age and activity and accommodated in 4 adjoining rooms of one building, as follows:

A, under or just over 1 year old, kept in cots;

B, aged 1 and 2 years;

C, aged 2 and 3 years;

D, aged 3 years.

These grades mixed very little, except that C and D sometimes played together out of doors; but the staff moved freely between the 4 rooms.

The play-school was in a separate building with different staff. It contained children aged 2–4 years, many of whom had formerly been in the nursery or had siblings there.

In October 1960 *H. influenzae* type *b* was isolated from 5 of the children in grades C and D (see Table 4). 13 female nursery staff were therefore swabbed 2 days later, but no type *b* strains were found. The nursery (but not the play-school) was

re-visited in March 1961, but of the 50 children present at that time only 22 had been examined in October 1960. These included only 2 of the 5 who had then yielded type *b* strains; neither of these did so again. However, both of the carriers of type *b* strains who were found in March had been present in October.

For the sake of simplicity, no reference has been made to the few capsulate strains of types other than *b* which were found in the groups of children already discussed. But at the second visit to the nursery 11 such strains were found (8 of type *e* and 3 of type *f*) among the 40 children of grades B, C and D, as compared with only one (of type *e*) on the first occasion. No capsulate strains were found among the babies of grade A on either occasion.

No illness attributable to *H. influenzae* was recognized among the nursery or play-school children during the relevant period.

The author's family

This consisted of 4 children, aged from 5 to 11 years, my wife and myself. All were swabbed in June 1960 and failed to yield *H. influenzae*. But in November 1960, when I was in frequent contact with the orphan home babies and their cultures, a swab from my own naso-pharynx yielded a type *b* strain, as did 3 others at approximately 3 week intervals until the beginning of January 1961. No *H. influenzae* was grown from the next swab, which was not collected until the end of February but a final specimen in the middle of March again yielded a type *b* strain. Meanwhile, the other members of the family, swabbed in November 1960 and in January and March 1961, failed to yield any type *b* strains despite my persistent carriage of such an organism and normal close family contacts.

DISCUSSION

The finding of a 41 % *H. influenzae* type *b* carrier-rate among the families of patients with haemophilus meningitis, as compared with rates of 2·4 and 2·9 % among the 'normal' groups, confirms the report from Yale (Good *et al.* 1943) that carriers are common in such families. But the meaning of this fact is far from clear. It is somewhat reminiscent of the situation described by Glover (1920) in connexion with meningococcal meningitis; but the two diseases are quite different, in that no epidemic of haemophilus meningitis has ever been reported. Seasonal fluctuations in incidence have been observed (Neal, Jackson & Appelbaum, 1934; Ouyang & Ting, 1957), and also groups of synchronous but geographically remote cases (Turk & Wynter, 1961); but there have been very few published reports of multiple cases occurring in the same household or institution, or otherwise demonstrably connected (Kline, 1962). Furthermore, the Yale workers' and my own findings do not permit the deduction that an abnormally high carriage-rate for *H. influenzae* type *b* precedes and gives rise to a case of meningitis. It could be that the affected child in the early stages of the disease is a potent source of infection for others in the household. It is unlikely that any one will ever have the good fortune to show which of these interpretations is correct by swabbing a family just before a case of haemophilus meningitis

occurs. But it may be possible to obtain retrospective information about the sequence of events by repeated measurements of type-specific antibodies in the blood of members of the family after the case has occurred. We are at present attempting to do this in Newcastle, using a serological procedure which had not been developed at the time of the Jamaican studies.

Ounsted (1950, 1951) found that children with haemophilus meningitis more often had 2 or more older siblings under the age of 12 years than did children of similar age admitted to hospital for other reasons, including meningococcal meningitis. He suggested that 'the *H. influenzae* organism requires one or two passages through partially immune contacts before a strain is evolved which can pass the meningeal barrier'. There are other possible explanations of his findings, as he admitted, and the good nutritional state of patients with haemophilus meningitis seen at the University College Hospital of the West Indies, as compared with most of those with other conditions, may indicate some influence of social status (Turk & Wynter, 1961). The sequence of events in the orphan home nursery, as shown in Table 3, would seem to have provided the ideal setting for Ounsted's evolutionary process, together with an exceptionally good supply of likely victims; and yet no case of meningitis occurred. It may be that only some strains of *H. influenzae* type *b* are even potentially pathogenic. The possibility must also be considered that the organism in the nursery was not in fact transmitted from child to child; for the babies were kept in well-spaced cots, and came into much closer contact with their attendants than with one another. Unfortunately permission to swab the staff could not be obtained. But if one of these had been found to carry *H. influenzae* type *b*, this would not have proved her to be the source of the babies' infection; for I was carrying such a strain during most of the time that I was visiting the nursery, and probably acquired it there, but I was clearly not the source from which 7 out of 10 children had been infected before my first visit there. Nor is it necessary to postulate a carrier among the staff to explain the rather high frequency of type *b* strains in the junior house of the orphan home; for although only the staff moved to and fro between the 2 groups of children, carriers of type *b* strains were promoted from the nursery to the junior house in September and October 1960, and this may also have happened earlier.

It seems unlikely that the high concentrations of capsulate strains found in grades B, C and D of the day-nursery were due to transmission by members of the staff; for no carriers were found among the staff in October 1960; when type *b* strains were common in grades C and D, and no capsulate strains were isolated on either occasion from the babies in grade A, who were attended by the same staff. It is rather surprising that the babies escaped infection, since several of them had older siblings in the other grades.

In the orphan home nursery there was always a time-lag before any new arrival became demonstrably infected with *H. influenzae* type *b* (see Table 3). Several of these new arrivals were too old for maternal antibodies to be invoked as an explanation of the delay, and it has been shown that very young babies can carry type *b* strains (Donald & Coker, 1957—quoted more fully later). The time-lag suggests that even in this nursery transmission of *H. influenzae* type *b* did not

occur readily. The failure of my own family to be demonstrably infected by my organism points in the same direction, but acquired immunity may have played some part there.

Development of immunity may also be the reason why some of the children in the orphan home nursery yielded type *b* strains on several occasions and then ceased to do so, but no serological investigations were carried out because none of the procedures then available seemed likely to give significant results. In almost every case the type *b* strain was succeeded by a non-capsulate strain (see table 3), a finding which accords well with the ideas of May (1958 and personal communications). He suggests that R (non-capsulate) strains found in chronic bronchitis may be partially degraded S (capsulate) strains that have lost their smooth colonial morphology during prolonged sojourn in the human body, but have retained traces of type specificity (detectable by gel-diffusion techniques) and some virulence.

Donald & Coker (1957) described an outbreak of a pertussis-like illness, associated with pneumonia in some cases, affecting 19 out of 30 infants in a nursery for premature babies. They recovered *H. influenzae* from the naso-pharynges of 21 infants, but not from any of the adult attendants. 8 of the 21 strains belonged to type *b*; it is not clear whether the rest were untypable or died before typing sera were obtained. The authors concluded that *H. influenzae* played a part in the pathogenesis of the respiratory illness, making the assumption that so high a carrier rate for this organism was abnormal. But is this true? Such a rate has not been reported by other workers, but it has seldom been looked for in appropriate communities. The findings in Jamaica indicate that a high carrier rate for *H. influenzae* type *b* in a group of small children is compatible with normal health and does not necessarily result in respiratory tract disease or meningitis. The circumstances in which this organism becomes pathogenic are as yet unknown.

SUMMARY

H. influenzae type *b*, the sero-type which causes meningitis, was isolated from 3 (2·4 %) of 127 naso-pharyngeal swabs from white non-Jamaican children resident in Jamaica, and from 14 (2·9 %) of 473 such swabs from a mixed group of Jamaican children. Much higher frequencies were found in households in which cases of haemophilus meningitis had recently occurred (41 %), in the nursery of an orphan home (up to 70 %) and in a day-nursery. In the orphan home nursery the high frequency persisted over a number of months, but no case of meningitis or other relevant disease occurred. A high concentration of this organism is therefore compatible with normal health, and the significance of high concentrations in the homes of meningitis patients is not certain.

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Salmonellae and shigellae in a group of periurban South African Bantu school children

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INTRODUCTION

From surveys by Bokkenheuser & Greenberg (1959) and Bokkenheuser & Richardson (1960) it is known that salmonella infections are frequent in South Africa. The role played by shigellae in causing serious infantile gastro-intestinal disorders was emphasized by Kahn (1957) and Kahn *et al.* (1958).

Investigating salmonella and shigella infections in a group of 124 rural Bantu school children, Bokkenheuser & Richardson (1960) found that, over a period of 1 year, nearly all the children were infected once, and many of them several times, by these pathogens. Clinical symptoms seldom accompanied the infections.

The present paper records a study of a similar group of Bantu school children living in a periurban area.

MATERIAL AND METHODS

The Witkoppen Bantu school, situated in open country 15 miles north of Johannesburg was chosen for this investigation. The school accommodated about 300 boys and girls mainly of the Iswana ethnic group. The children were generally well dressed and in fair nutritional condition as judged by height and weight for the particular age-groups. Most of them lived nearby, although some came from homes as far as 6 miles away. The parents were mainly semi-skilled or unskilled labourers, e.g. farm workers, tractor drivers, builders or painters. Few families kept livestock (cattle, goats, pigs, chickens) but most grew some maize or vegetables, failing which these products were obtained from the local trading store. The chief elements of the diet were maize porridge and bread with occasional consumption of kaffir corn (*sorghum vulgare*), tea, coffee and milk. Vegetables together with wild spinaches (*morogo, m'fino*) were eaten according to season. In a few families meat was consumed daily, but in most only two to three times a week. Eggs, butter and fish were rarities. During attendance at school the children received one meal daily, usually 'poosa-munda' (dried fermented porridge reconstituted with water) and bread. Briefly, the diet was possibly adequate in energy value, high in carbohydrate, low in animal protein and fat as well as in calcium and certain vitamins.

The water was drawn from bore-holes, shallow surface-wells, rain-water tanks, rivers or streams.

To match the previous study (Bokkenheuser & Richardson, 1960) a group of 128 children aged between 7 and 18 years was selected. During the year of observation no alteration was made in their mode of life. A few occasionally attended a Bantu health clinic about $\frac{1}{4}$ mile from the school, but in one instance only for a gastro-intestinal disorder which, as far as could be ascertained, was not treated with antibiotics. It is not known whether the children were treated by witch-doctors or given tribal remedies including purgatives.

Over a period of 1 year, the children were examined eight times at fairly regular intervals and their general condition and oral temperatures were recorded. The consistency of the stools was noted, and the specimens were planted on SS-agar and selenite-F media within 10 min. of voiding. Plates and enrichment media were taken to the laboratory in Johannesburg and incubated. The following morning a loopful of the enriched culture was planted on SS-agar and incubated. If present, 3 lactose-negative colonies were transferred from each plate to composite media for biochemical reactions. The antigenic structure of salmonellae was determined in detail whereas the shigellae were classified by their principal group antigen.

The pathogenic organisms were tested for sensitivity to a range of antibiotics. Disks, 6 mm. in diameter and weighing 3·3 mg., were impregnated with the following antibiotics: penicillin G (10 units), streptomycin (100 μg.), erythromycin (50 μg.), chloramphenicol (50 μg.), tetracycline hydrochloride (50 μg.), neomycin (50 μg.) and novobiocin (50 μg.). The inhibitory activity of these disks was checked in parallel on blood agar plates, using *Staphylococcus aureus* (Oxford strain) as the control. A zone of inhibition of less than 2 mm. was taken to indicate a resistant organism.

RESULTS

In the group of 128 children the younger age-groups and females predominated slightly (Table 1). Of 891 faecal specimens, 54 (6·1 %) yielded a growth of salmonellae and 6 (0·7 %) of shigellae. The salmonellae comprised 18 serotypes and the shigellae 2 (sonne and flexner). During the year of observation 31·3 % of the children experienced either salmonellosis or shigellosis or both. Of these, 37 (28·9 %) were infected with salmonellae and 6 (4·7 %) with shigellae. The infection rates appeared slightly higher among females and in the younger age-groups.

Because some children were absent on the days of examination and others left school before the end of the survey, only 75 children were available for all eight investigations. Of these, 22 (29·3 %) yielded a growth of salmonellae and 2 (2·7 %) of shigellae. Ignoring for a moment infections of long duration and the question of reinfections, it emerges from Table 2 that salmonellae were recovered from 1·3–9·3 % of the children the year round and that no distinct outbreak was encountered. Although *Salmonella labadi* was the commonest salmonella type found, the striking feature was the sporadic occurrence of many different serotypes.

A total of 35 strains was recovered from these children. Double infection of specimens was not observed. In 14 of the 22 children, however, 2–4 different types were isolated during the observation period. In some cases the same salmonella

Table 1. *Salmonella and shigella infections by age and sex*

Age (years)	Number of individuals	Number of specimens	Specimens infected with: Salmonellae No.	Specimens infected with: Salmonellae %	Specimens infected with: Shigellae No.	Specimens infected with: Shigellae %	Individuals infected with: Salmonellae No.	Individuals infected with: Salmonellae %	Individuals infected with: Shigellae No.	Individuals infected with: Shigellae %	All infected individuals No.	All infected individuals %
7–10	67	474	31	6·5	4	0·8	22	32·8	4	6·0	24	35·8
11–18	61	417	23	5·5	2	0·5	15	24·6	2	3·3	16	26·2
Total	128	891	54	6·1	6	0·7	37	28·9	6	4·7	40	31·3
Females	73	508	37	7·3	3	0·6	23	31·5	3	4·1	26	35·6
Males	55	383	17	4·4	3	0·8	14	25·5	3	5·5	14	25·5

Table 2. *Seasonal distribution and duration of salmonella and shigella infections in 75 children*

Month of examination	Recovered salmonellae: *S. montevideo*	*S. derby*	*S. newport*	*S. london*	*S. labadi*	*S. typhimurium*	*S. anatum*	*S. thompson*	*S. wegbridge*	*S. paratyphi A*	*S. johannesburg*	*S. adelaide*	*S. chester*	*S. pomona*	Percentage salmonella-infected individuals	Recovered shigella	Percentage shigella-infected individuals	Total infected individuals No.	Total infected individuals %
March	1	1	2	—	—	—	—	—	—	—	—	—	—	—	5·3	—	—	4	5·3
April	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1·3	1	1·3	2	2·7
June	—	—	—	—	2	1	1	—	—	—	—	—	—	—	5·3	—	—	4	5·3
July	—	—	—	—	1	—	—	2	—	—	—	—	—	—	4·0	—	—	3	4·0
August	—	—	—	1	1	—	1	—	2	1	—	—	—	—	8·0	—	—	6	8·0
October	—	—	—	—	1	1	—	—	—	—	1	1	2	1	9·3	—	—	7	9·3
November	—	—	—	—	2	1	1	—	—	—	—	—	2	—	8·0	—	—	6	8·0
January	—	1	—	—	1	2	—	—	—	—	—	—	—	—	5·3	1	1·3	5	6·7
Total	1	2	2	2	8	5	3	2	2	1	1	1	4	1	—	2	—	—	—

type was isolated several times from the same individual (Table 3). Some types (*S. johannesburg*, *S. typhimurium* and *S. thompson*) were recovered in successive samples, while others (*S. labadi* and *S. anatum*) reappeared in the same individual with longer intervals. In half of these children the temperatures were normal (98·4° F.) throughout, whereas in the other half the temperatures were raised by 1° F. at those times when their stools yielded pathogens. The faeces were usually semi-solid to hard, only two specimens were soft, none was liquid. In agreement with this the children appeared to be in good health.

Table 3. *Repeated recovery of the same salmonella type from indivual children*

Number of child	Time of collection					
	June	July	August	October	November	January
6	La	—	—	—	La	—
7	La	—	La	—	La	—
100	—	La	—	La	—	La
30	Ch	—	—	Ch	—	—
62	—	—	—	Ch	Ch	—
64	—	—	—	Jo	Jo	—
79	—	—	Tm	Tm	—	—
99	An	—	An	—	An	—
120	—	—	—	Th	Th	—

La, *Salmonella labadi*; Ch, *S. chester*; Jo, *S. johnannesburg*; Tm, *S. typhimurium*; An, *S. anatum*; Th, *S. thompson*.

Table 4. *Clinical observations related to infection*

	Number of specimens	Consistency of faeces					
		Hard		Soft		Liquid	
		No.	%	No.	%	No.	%
Pathogens not recovered	831	289	35	533	64	9	1
Salmonella infected	54	23	43	30	56	1	2
Shigella infected	6	1	17	3	50	2	33
Total infected	60	24	40	33	55	3	5
		Temperature (°F.)					
		< 98·4		98·4–99·8		100+	
		No.	%	No.	%	No	%
Pathogens not recovered	831	317	38	414	50	100	12
Salmonella infected	54	13	24	33	61	8	15
Shigella infected	6	1	17	4	67	1	17
Total infected	60	14	23	37	62	9	15

Taking all infected individuals into account (Table 4) one is impressed by the mildness of the condition. Diarrhoea was infrequent and, as compared to the controls, there was no evidence of the pathogens affecting the consistency of the stools. Furthermore, the infections were not associated with a marked rise in

temperature. It is noteworthy that during the year none of the children gave a history of diarrhoea.

The salmonella and shigella strains were tested for sensitivity to a range of antibiotics. On occasions where several colonies of the same type were isolated from a specimen, they were all examined and the average sensitivity recorded. As might be expected (Table 5), both salmonellae and shigellae were generally resistant to penicillin and novobiocin. But, in addition, the salmonellae showed considerable resistance to erythromycin and some to chloramphenicol and tetracycline.

Table 5. In vitro *resistance to antibiotics of isolated strains*

		Percentage of strains resistant to						
Genus	Number of strains	Penicillin	Strepto-mycin	Erythro-mycin	Chloram-phenicol	Tetra-cycline HCl	Neo-mycin	Novo-biocin
Salmonella	51	84·3	0	62·7	3·9	2·0	0	100
Shigella	6	100	0	0	0	0	0	100

Table 6. *Origin and quality of drinking water*

	Escherichia coli/100 ml.						Colony count per ml.	
	Faecal			Presumptive				
	0	1–4	> 4	0	1–10	> 10	< 100	> 100
Origin of water	Number of specimens							
Well (machine pump)	4	1	—	1	1	3	—	5
Well (windmill)	4	—	1	3	—	2	1	4
Surface well	—	—	8	—	—	8	—	8

Because drinking water might be involved in the transmission of the infection, inquiries were made into its origin and quality. The water at the school was drawn by hand-pump from an enclosed well. On repeated examinations the total number of colonies developing per ml. ranged from 3–53. Presumptive *Escherichia coli* ranged from 0–2 per 100 ml., and faecal *E. coli* was absent. In view of the reasonably satisfactory quality of the school water, samples were obtained from the homes, which drew their supply from different sources (Table 6). While the machine and windmill water was of mediocre quality, the water drawn from the surface wells was decidedly unsatisfactory (South African Bureau of Standards, 1951).

DISCUSSION

The results of incidence surveys of infections depend on a number of factors (Bokkenheuser & Richardson, 1960) among which the most important are: (*a*) the number of samples investigated; (*b*) the time-lag between collection and planting of the specimens; (*c*) the selection of a suitable portion of the specimen; (*d*) the fastidiousness of the micro-organisms; (*e*) the media employed; and (*f*) the

number of colonies examined from each specimen. It follows, therefore, that statistically significant differences in the recovery rates of two pathogens do not necessarily reflect corresponding differences in incidence of infection. Moreover, it is almost impossible to establish the incidence of mild, benign infections unless specimens of faeces are examined very frequently. With these reservations, it may be stated that both shigella and salmonella infections occurred among the Witkoppen school children and that at least salmonella infections were quite frequent. Infections were observed at all times of the year but no outbreak was encountered.

The infected children showed no clinical signs of illness and with one or two exceptions there was no history of acute or chronic gastro-intestinal disturbances. A striking observation was the reappearance of certain salmonellae in the same individuals. Although it may be that these children (Table 3) were reinfected once or twice with the same serotype, it is more likely that some of them were carriers of long standing.

The poor quality of the water, particularly that drawn from surface wells, made it highly probable that drinking water was involved in the transmission of the infections.

Information from this survey and that from the Tlaseng Bantu school children (Bokkenheuser & Richardson, 1960), obtained with the same technique, tend to supplement each other. Salmonella and shigella infections occurred in both groups and it is likely that nearly all the children experienced one, and many of them several, infections annually by these pathogens. The infections occurred throughout the year and were usually asymptomatic. While there was evidence of a periodic increase in incidence in the Tlaseng study, it is conceivable that carriers of salmonellae were present among the Witkoppen children. In both groups the quality of the drinking water was very poor and might well be incriminated in the transmission of the infections. The salmonella strains isolated from the Tlaseng children in 1958–59 were highly sensitive to erythromycin and chloramphenicol. In contrast, the strains recovered 2 years later from the Witkoppen children were highly resistant to erythromycin and showed evidence of increasing resistance to chloramphenicol. Their frequency of resistance to chloramphenicol and tetracycline corresponded to the rates reported from Holland (Manten, Kampelmacher & Guinee, 1961).

Although salmonella and shigella infections were extremely benign among African school children, it does not follow that they do not take their toll among the most susceptible groups—the infants and the aged.

SUMMARY

1. Faeces from apparently healthy Bantu school children of a periurban district of Johannesburg were examined eight times at regular intervals over a period of 1 year.

2. Of 75 children, 29·3 % experienced at least one salmonella infection and 2·7 % one shigella infection. It is suggested that over a year nearly all children will have one, and many of them several, infections with these pathogens. The infections occurred at a low rate throughout the year.

3. In most cases the infections were asymptomatic. A few of the children showed evidence of being salmonella carriers of long standing.

4. Eighteen different salmonella types were recovered. *Salmonella typhi*, *S. paratyphi* B and *S. paratyphi* C were absent. The organisms were highly resistant to penicillin, erythromycin and novobiocin and a few strains were also resistant to chloramphenicol and tetracycline.

5. The drinking water was of poor quality and may well be implicated in the transmission of the infections.

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