A GUINEA-PIG EPIZOOTIC ASSOCIATED WITH AN ORGANISM OF THE FOOD-POISONING GROUP BUT PROBABLY CAUSED BY A FILTER-PASSER.

By G. F. PETRIE and R. A. O'BRIEN.

(*From the Lister Institute of Preventive Medicine.*)

CONTENTS.

https://doi.org/10.1017/S0022172400042960 Published online by Cambridge University Press

Introductory[1].

DURING the past winter we have had an opportunity of studying two epizootics which broke out amongst the stock guinea-pigs in the Lister Institute, Chelsea. The first occurred in a room in which were nearly 200 guinea-pigs. This epidemic began in the third week of October and progressed so rapidly that by the second week of November all the animals had died. The second epidemic affected a stock of about 300 animals which were housed in a different room in the animal house, the mortality in this case being about 90 per cent. This outbreak occurred in the first week of November and lasted about five weeks. All the animals in both stocks were young, weighing on an average about 250 grammes.

In both instances the animals were allowed to run freely about the floor of the rooms. Sick animals showed a roughening of the fur, and when placed on the side were unable to regain the upright position apparently owing to a weakness of the limb muscles.

The post-mortem appearances varied a good deal. The intestines frequently showed congestion. The liver and spleen also were usually congested and occasionally contained small grey or yellow nodules. In some of the cases the suprarenals showed varying degrees of congestion, and patchy congested areas were often seen in the lungs. Effusions into the serous cavities did not occur.

Characters of the bacillus isolated.

Cultures made from the organs either after direct plating on lactose bile salt agar or after preliminary incubation in dulcite bile salt broth gave, in a large proportion of the cases, pure growths of a bacillus which from its cultural characteristics evidently belonged to the paratyphoid group of organisms. This bacillus has been identified as being closely related to the *B. suipestifer* (the hog-cholera bacillus) and to the *B. aertryck*: indeed it would appear that these three organisms are indistinguishable by every known test including those of agglutination and absorption. The organism was isolated most easily from the contents of the small intestine. Thus it was recovered in pure culture in 12 out of 16 examinations. It was generally easy to obtain cultures from the faeces of the epizootic stock animals, at least three or four days before the death of the animals. The

[1] For the sake of brevity, extended references to the details in the Tables have been omitted in the text.

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liver and spleen yielded cultures of the bacillus and less frequently it was obtained from the heart-blood. When present in the heart-blood the colonies on the plates were not numerous—a marked septicaemia did not appear to exist. On three or four occasions in which the bacillus was isolated from the faeces three or four days before death, cultures from the heart-blood and liver failed to give positive results. The bacillus was isolated from the bile once, but not at all from the urine in five cases examined.

Pathogenicity experiments.

The pathogenicity of the bacillus was tested on guinea-pigs, rats, mice and rabbits. The bacillus is extremely pathogenic to guinea-pigs when given subcutaneously but does not readily kill them when given per os. The results of pathogenicity tests are exhibited in Table I. Shortly stated these results are as follows. For guinea-pigs of 250 grms. weight doses varying from 0·0001 to 0·000001 c.c. of a young broth culture inoculated subcutaneously proved fatal in some instances only, but doses of 0·001 c.c. invariably caused death in about five days.

A dose of 2 c.c. given subcutaneously to white rats was fatal in six days, while 1 c.c. inoculated intraperitoneally killed in 24 hours.

A dose of 0·01 c.c. either intraperitoneally or subcutaneously proved fatal to mice in two days.

When using rabbits we found that 0·1 c.c. injected subcutaneously killed an animal of 900 grms. in two days, 0·1 c.c. intraperitoneally killed a rabbit of 1300 grms. in 24 hours, and 0·01 c.c. given intravenously killed in five days, the weight of the animal being 1300 grms.

Post-mortem appearances of guinea-pigs inoculated subcutaneously.

The local reaction is the principal feature post-mortem, there being in acute cases intense haemorrhagic oedema, in those dying later local necrosis or abscess. It was rare to find even minute spots in the spleen and liver, and it seems doubtful whether by subcutaneous inoculation or feeding one can produce the yellowish white nodules seen in the epizootic animals. There were no other constant features in the artificially inoculated animals.

TABLE I.

Showing results of pathogenicity tests.

Animal used	Dose of culture	Method of inoculation	Result	Cultures	Remarks
Guinea-pigs	0·000001 c.c. 24 hr. broth culture	Subcutaneous	Died, 8 days	Heart blood +	—
	0·000001 ,, ,, ,,	,,	Died, 25 days	—	—
	0·00001 ,, ,, ,,	,,	Died, 8 days	Local lesion +	—
	0·00001 ,, ,, ,,	,,	Survived	—	—
	0·0001 ,, ,, ,,	,,	Died, 3 days	H.B., Local lesion +	Two experiments.
	0·0001 ,, ,, ,,	,,	Survived	—	—
	0·001 ,, ,, ,,	,,	Died, 5 days	H.B. +	Days lived = average of five expts.
	0·01 ,, ,, ,,	,,	Died, 4 days	H.B. +	,, ,, ,, ,,
	0·02 ,, ,, ,,	,,	Died, 2 days	—	—
	0·1 ,, ,, ,,	,,	Died, 3 days	H.B. +	Days lived = average of three expts.
	1 ,, ,, ,,	,,	Died, 2 days	—	—
White rats	0·1 c.c. 48 hr. broth culture	Subcutaneous	Survived	—	—
	1·0 ,, ,, ,,	,,	Survived	—	Necrosis at site of injection.
	2·0 ,, ,, ,,	,,	Died, 6 days	H.B. & Liver +	—
	0·1 ,, ,, ,,	Intraperitoneal	Survived	—	—
	1 ,, ,, ,,	,,	Died, 1 day	H.B., Liver, peritoneal effusion +	—
Mice	0·01 c.c. 24 hr. broth culture	Subcutaneous	Died, 2 days	H.B. +	—
	0·02 ,, ,, ,,	,,	Died, 2 days	H.B. +	—
	0·1 ,, ,, ,,	,,	Died, 1 day	H.B., Liver, Small intestine +	—
	0·01 ,, ,, ,,	Intraperitoneal	Died, 2 days	H.B. +	—
	0·02 ,, ,, ,,	,,	Died, 12 days	H.B. +	—
	0·1 ,, ,, ,,	,,	Died, 4 days	H.B. +	—
Rabbits	0·1 c.c. 24 hr. broth culture	Subcutaneous	Died, 2 days	H.B. +	Wt. 900 grms.
	0·5 ,, ,, ,,	,,	Died, 4 days	H.B. +	,, 1100 ,,
	1 ,, ,, ,,	,,	Died, 8 days	H.B., Liver +	,, 1150 ,, Marked diarrhoea.
	2 ,, ,, ,,	,,	Survived	—	,, 1200 ,, Local necrosis.
	0·1 ,, ,, ,,	Intraperitoneal	Died, 1 day	H.B., Liver +	,, 1300 ,,
	0·5 ,, ,, ,,	,,	Survived	—	,, 1400 ,,
	0·01 ,, ,, ,,	Intravenous	Died, 5 days	H.B., Liver +	,, 1300 ,,
	0·02 ,, ,, ,,	,,	Died, 8 days	Liver +	,, 1500 ,, Diarrhoea.
	0·1 ,, ,, ,,	,,	Died, 4 days	—	,, 1300 ,,

+ = the bacillus recovered.

References to epizootics associated with bacilli of the food-poisoning group.

References in the literature to guinea-pig epizootics associated with this bacillus are scanty. Kovárzik (1903) has given an account of an epizootic amongst guinea-pigs in Budapest which he ascribed to a variety of *B. coli.* Lehmann and Neumann, however, include the bacillus in the Gaertner group. MacConkey (1905) notes that an organism indistinguishable from *B. enteritidis* (Gaertner) seemed to be the cause of an epidemic amongst the experimental guinea-pigs at the serum department of the Lister Institute near London. One of us (G. F. P.) some years ago observed an epizootic amongst young guinea-pigs in the laboratory stock in Bombay associated with an organism giving the cultural reactions of the Gaertner group. The disease occurred in the monsoon season and affected only young animals. Eckersdorff (1908) has given a brief description of a guinea-pig epizootic in Frankfurt-a-Main associated with a bacillus of the paratyphoid group.

Filtration experiments.

There can be no doubt as to the close association of the bacillus described above with the epizootic disease. But the disease evidently presents some analogies with swine fever, a disease which has been proved to be due to a filter-passing virus,—the associated *B. suipestifer* so frequently cultivable from the organs being merely a secondary invader of no etiological importance. Dr F. A. Bainbridge carried out some filtration experiments with the organs of infected guinea-pigs in this epizootic, but was unable to pursue the investigation. The details of our experiments dealing with this point are displayed principally in the tables, but we may comment here on the experiments as a whole.

The first experiment we carried out, although not a filtrate experiment, gave results of such significance that it was considered worth while to experiment with the filtered organs of the epizootic animals. The experiment consisted firstly in sowing 0·003 c.c. of heart-blood from each of seven guinea-pigs which had died of the epizootic disease into dulcite-broth—a medium which is known to be of especial service in the detection of organisms of the paratyphoid group.

The culture tubes from three of them gave a growth of the bacillus and further results from these animals are not included in the table.

Table II. shows the results of subcutaneous inoculation into each of two guinea-pigs of minute quantities (0·0003 c.c) of the four samples of heart-blood giving negative cultures. All these animals died in periods varying from 7 to 14 days.

TABLE II.

Experiments in which minute amounts of heart-blood from infected guinea-pigs were inoculated into healthy guinea-pigs.

No. of guinea-pig	Dose of blood inoculated	Cultures from H.B. and intestines	Days lived
1	0·0003 c.c. subcutaneously	Negative	12
1 *a*	,, ,,	,,	14
2	,, ,,	,,	9
2 *a*	,, ,,	,,	15
4	,, ,,	H.B. = negative Intestines = positive	9
4 *a*	,, ,,	Negative	12
6	,, ,,	,,	7
6 *a*	,, ,,	,,	14

In this experiment the objection may be raised that the inoculation test is usually a more delicate one than the cultural when a pathogenic bacillus is present in small numbers, and that these guinea-pigs may have died as the result of an infection by the bacillus in spite of the negative cultures. Against this view is the fact that the bacillus was isolated after death from only one of the eight animals. Moreover there was not present in any of the dead guinea-pigs the characteristic local lesion which invariably follows the inoculation of cultures of the bacillus subcutaneously.

We must regard this experiment then as strongly suggesting that some organism other than the *B. suipestifer* was responsible for the deaths of the inoculated animals.

Methods used in the filtration experiments.

The filtrates used in the experiments were obtained by first of all grinding up the organs—generally the spleen, liver, lungs and heart with sterile sand in a sterilised mortar. Sterile salt solution was added in varying amounts during this process and subsequently, the object being to secure an extract of the organs in saline of such a consistency that the filtration of the fluid through the Berkefeld filter should be fairly rapid and completed within one hour. The amount of saline

added to the organs of each guinea-pig varied from 40 to 120 c.c. The material after grinding was filtered through a Buchner's filter connected up with a vacuum pump. This filtrate was finally filtered through the sterilised Berkefeld filter. The sterility of the filtrates was tested in every instance by sowing 3 or 4 c.c. into dulcite bile salt broth and into ordinary peptone broth.

Comments on filtrate experiments.

Table III. In this series a moribund pig from the epizootic stock was killed and the organs ground up and filtered. The post-mortem appearances were typical and the bacillus was isolated from the contents of the small intestine. It will be seen that a large proportion of the guinea-pigs inoculated by various methods (subcutaneous, intraperitoneal, intracerebral, intravenous, intranasal and feeding) with the filtrate died. Special attention may perhaps be directed to the three animals fed with the filtrate and the three inoculated nasally, since these two methods, as in the case of swine-fever, are probably natural modes of infection.

From the table it will be seen that the filtrate experiments were carried on serially, the filtrates of dead animals being in their turn used for further inoculation experiments, and so on. Some indications are met with of a diminution of virulence of the infecting agent as a result of passage. A similar phenomenon has been noted by Dorset in his experiments with the filter-passer of swine fever.

In addition to the experiments with the filtrates of organs of epizootic guinea-pigs, experiments with filtered serum, bile and urine were performed and the results are recorded in Table IV.

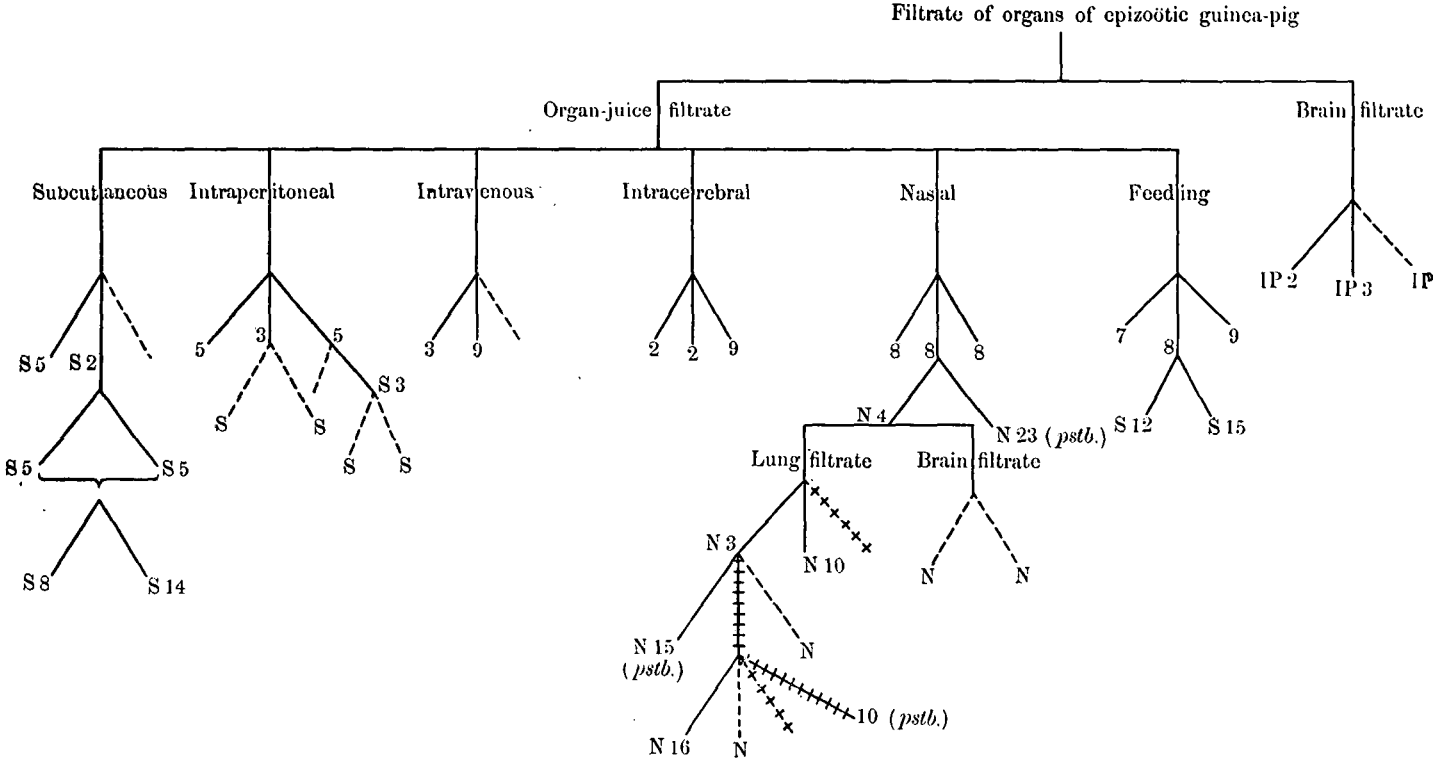
Table V. This table summarises experiments with the filtrates of organs from several of the animals which died as a result of the inoculation of minute amounts of blood from guinea-pigs dying during the epizootic (vide supra).

Post-mortem appearances and culture results of experimental animals.

The only fairly constant pathological feature met with post-mortem was a reddening of the wall of the small intestine.

In the animals inoculated intracerebrally with 0·3 c.c. of the filtrate the brain showed extreme congestion. Marked congestion of the lungs was found in the guinea-pigs inoculated nasally with the original filtrate (see Table III.).

TABLE III.



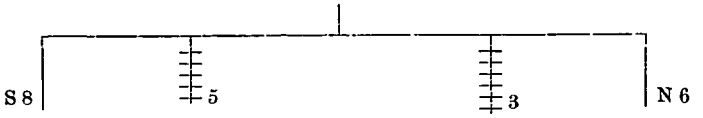
Signs used in this and the following tables:

------- = inoculated animal survived.	S = inoculated subcutaneously.
——— = inoculated animal died.	N = inoculated nasally.
+++++++ = contact survived.	IP = inoculated intraperitoneally.
+++++++ = contact died.	*pstb.* = pseudotuberculosis found p.-m.

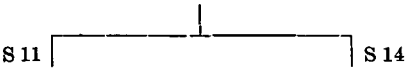
Numbers represent "days lived."

TABLE IV.

Filtrate of urine collected from eight dead epizootic guinea-pigs, and diluted 1 in 10 with saline solution.



Filtrate of diluted bile.



Filtrate of diluted serum from eight guinea-pigs observed to be sick and killed.

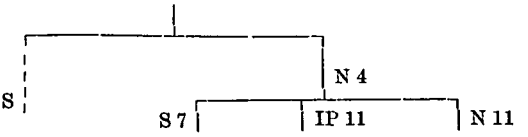
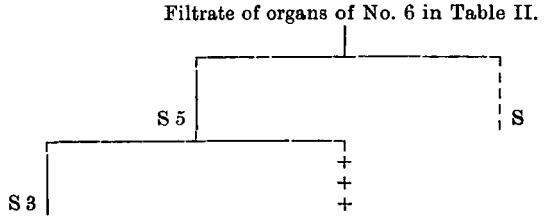
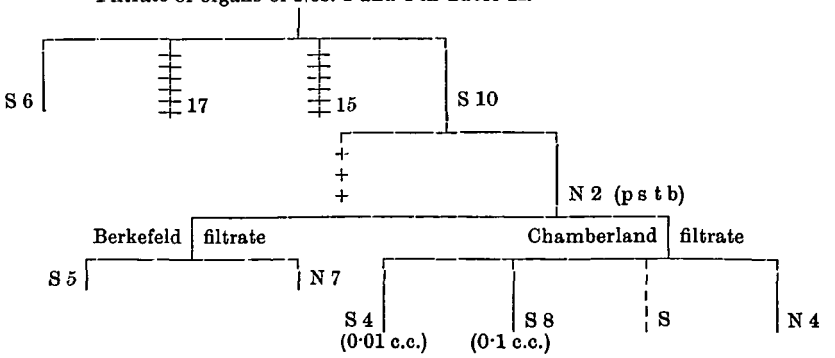


TABLE V.

Filtrate of organs of No. 6 in Table II.



Filtrate of organs of Nos. 2 and 4 in Table II.



https://doi.org/10.1017/S0022172400042960 Published online by Cambridge University Press

The feeding experiments in which 60 c.c. of the filtrate were mixed with the food of three guinea-pigs may be summarised thus. Animal No. 1 (see Table III.) died in seven days, with congestion of the liver, pancreas and upper part of the small intestine. Cultures from the liver, heart-blood and small intestine gave no growth of the bacillus.

Animal No. 2 died in eight days, with congestion of the liver, small intestine and lungs; the bacillus was not found in the contents of the small intestine or in the heart-blood.

Animal No. 3 died in nine days, with congestion of the intestines, the heart-blood and intestinal contents giving no growth of the bacillus.

Cultures. In only three of 49 animals dying after the inoculation of filtrates was the bacillus recovered, although in every case the heart-blood and intestinal contents were sown into dulcite bile salt broth.

Attempts to cultivate a virus from the heart-blood and from the filtrates of organs.

The media used for growing this hypothetical virus were mixtures of agar with guinea-pig blood, guinea-pig serum, and filtrates of the organs of normal guinea-pigs. To these were added varying minute amounts of the heart-blood of recently dead guinea-pigs from the infected stock and of filtrates of the organs of infected pigs. The cultures were kept for two to three weeks at 37° C., some aerobically and some anaerobically. Two of these cultures were examined by the "dark-ground" method of illumination and showed innumerable extremely minute bodies with very active Brownian movements. The appearances exactly resembled those described by Flexner and Lewis in their culture tubes of the virus of anterior poliomyelitis. In several instances the heart-blood taken directly from dead animals was examined by this method, but showed nothing unusual nor did control uninoculated culture tubes contain any of these minute bodies.

Four of the culture tubes to which the filtrate of the organs of the animal furnishing the material for the experiments in Table III. was added were incubated for a fortnight aerobically, and from them varying amounts of fluid were drawn off and used for subcutaneous inoculation. Nine of the twelve inoculated guinea-pigs died (see Table VI.), animals 1 to 8 being inoculated from one culture tube; of these six died after periods roughly proportional to the dosage. Of the four guinea-

pigs inoculated with material from the remaining tubes only one survived. These experiments are interesting, but do not of course necessarily prove any multiplication of the virus in the culture tubes.

TABLE VI.

Showing results of inoculation into guinea-pigs of contents of culture tubes containing organ-juice filtrates.

No. of guinea-pig	Dose	Culture inoculated	Result
1	0·001 c.c.	296 *d*	Survived.
2	,,	,,	Died in 10 days.
3	0·01 c.c.	,,	Survived.
4	,,	,,	Died in 19 days.
5	0·1 c.c.	,,	Died in 5 days.
6	,,	,,	,, ,,
7	1 c.c.	,,	Died in 3 days.
8	,,	,,	,, ,,
9	2 c.c.	296 *a*, *b*, and *c*	Died in 10 days.
10	,,	,, ,,	Survived.
11	3 c.c.	,, ,,	Died in 19 days.
12	,,	,, ,,	Died in 26 days.

Control experiments.

A number of control experiments was done, animals being injected with the filtrates of organs of apparently healthy stock animals and with the filtrates of organs of animals killed by subcutaneous inoculation of the bacillus. Unfortunately the whole series was vitiated by the sudden appearance of *B. pseudo-tuberculosis rodentium*. Several animals amongst the Institute stock at this time died with the post-mortem appearances of this disease and *B. pseudo-tuberculosis rodentium* was recovered from them. A considerable number of the control animals also died of this disease. Fortunately this complication did not occur until after the completion of most of the filtrate experiments.

We consider it unnecessary to burden our paper with full details of these control experiments, since we ourselves have found it impossible to draw any clear deductions from them.

We may add that several filtrates prepared from epizootic material were kept six weeks in the cold room and were then retested by subcutaneous inoculation on healthy guinea-pigs. Very few of the inoculated animals died, apparently showing that the filtrates had lost their virulence.

Discussion as to the causal agent of the disease and its transmission—Bacillus or Filter-passer?

The relation of the bacillus to the epizootic.

Before we can accept a filter-passer as the essential infective agent, it must be shown that the associated bacillus is in itself incapable of spreading the disease. Now if we assume that this bacillus is the causal agent of the disease, it must be supposed that the natural mode of infection is either subcutaneous or per os.

I. *Possible entry of the bacillus through the skin.*

The bacillus might be inoculated by lice, for guinea-pigs are infested with lice and with no other parasites; but against transmission by lice is the fact that the septicaemia when it did occur in naturally infected animals did not appear to be a marked one, so much so that in a considerable proportion of cases the bacillus could not be cultivated from the blood. Thus, out of cultures made from the heart-blood of fourteen epizootic guinea-pigs only three were positive.

The following experiments bearing on the question of transmission by lice were carried out:

1. A healthy stock guinea-pig was tied down in a tray and the corpse of a guinea-pig which died during the epizootic and which was covered with lice was left in contact with it for five minutes. The lice certainly transferred themselves, for they were seen attached to the ends of the hairs of the stock animal after the corpse was removed, a position in which they are never seen under ordinary circumstances in the case of a living guinea-pig. The stock guinea-pig was kept under observation for three or four months but showed no symptoms indicating an infection.

2. Some lice from the fur of a guinea-pig dying two days after the subcutaneous inoculation of one c.c. of a broth culture of a strain of the bacillus recovered during the epizootic were transferred directly to a healthy guinea-pig. The animal survived.

It may be added that no appearance was found in the animals dead of the disease which suggested that the bacillus gained entry through the skin. In animals artificially inoculated subcutaneously there is found an intense haemorrhagic oedematous infiltration at the site of injection.

II. *Possible transmission of the bacillus by feeding.*

Attempts were made to infect animals by feeding them with broth cultures of the bacillus, in some cases a single contaminated meal being given, in others several doses of culture and in one series massive doses were repeatedly given with food (Table VII.). On the whole these experiments confirm the observations of various workers, that feeding guinea-pigs with bacilli of the paratyphoid group is an uncertain method of infection.

The bacillus was recovered from about one-third of the pellets of faeces picked from the floor of the room in which the infected stock pigs were confined, so that it may be supposed that opportunities for the contamination of food with the bacillus were numerous, but the uncertainty of feeding as a mode of infection above referred to considerably lessens the significance of these observations in relation to the natural spread of the disease.

The bacillus was not recovered from the dust collected from crevices a short distance above the floor.

III. *Possible transmission of the bacillus by contact with infected animals.*

Healthy guinea-pigs were put into the same cages with animals inoculated subcutaneously with varying doses of the bacillus and with others fed with the bacillus, but few of them died and from them the bacillus was rarely recovered (Tables VII and VIII.).

In this connection it is worth remembering that the intestinal contents of animals subcutaneously inoculated with fatal doses of the bacillus almost invariably contained the bacillus, and often in pure culture.

TABLE VII.

Showing the results of feeding experiments and of putting healthy guinea-pigs as contacts with the fed animals.

No. fed	Dose of cultures	Result	Cultures from fed guinea-pigs	No. of contacts	Result	Cultures from contact guinea-pigs
3	12 c.c. 4 days broth culture each	All survived	——	6	All survived	——
4	One 3 days agar slope each	Killed 49 days later	Faeces of all 9 days later positive. Small intestines and organs when killed all negative	8	All survived	——
1	6 c.c. 24 hrs. broth cult. on 3 occasions	Survived	Faeces + 5 wks. after last feeding	2	Both survived	——
1	,, ,,	,,	Faeces + 22 dys. after last feeding	2	Both survived	——
1	,, ,,	,,	Faeces + 5 wks. after last feeding	2	1 died, 1 survived	H.B., Liver, Intestine, all negative.
1	,, ,,	,,	Faeces + 3 wks. after last feeding	2	1 died, 1 survived	H.B., Intestine, negative; Spleen positive.
1	,, ,,	,,	Bacillus not recovered in faeces	2	1 died, 1 survived	H.B., Liver, Intestine, all negative.
1	,, ,,	Died in 4 days	Liver and intestine +	—	—	——
2	6 c.c. 48 hrs. broth culture each	Both survived	——	4	2 survived, 2 died	– Cultures negative.
1	,, ,,	Died	Liver and intestine positive	2	2 survived	—–
11	60 c.c. 24 hrs. broth culture	All survived	——	0	—	——
4	,, ,,	Died in 13 days	H.B. of all positive	0	—	——
1	,, ,,	Died in 5 days	H.B. = 0	0	—	——
1	,, ,,	Died in 6 days	——	0	—	——
1	,, ,,	Died in 7 days	H.B. = 0	0	—	——
1	,, ,,	Died in 14 days	H.B. = +	0	—	——
1	,, ,,	Died in 15 days	H.B. and intestines = 0	0	—	——

TABLE VIII.

Showing results of subcutaneous inoculation of the bacillus into guinea-pigs together with the results of contact experiments with the inoculated guinea-pigs.

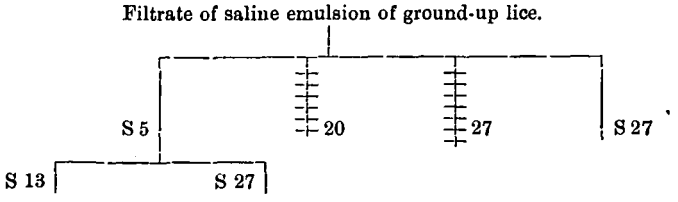
No. of guinea-pig	Dose	Days lived	Culture from small intestine	No. of contacts used	No. of contacts surviving
1	0·1 c.c.	4	Bacillus recovered	2	2
2	,,	2	—	2	2
3	0·01 c.c.	4	Bacillus recovered	2	2
4	,,	3	,,	2	1
5	,,	7	,,	2	2
6	0·001 c.c.	7	,,	2	2
7	,,	7	,,	2	2
8	,,	5	,,	2	2

THE RELATION OF THE FILTER-PASSER TO THE EPIZOOTIC.

I. *Possible entry of the virus through the skin.*

The bodies of two guinea-pigs dying during the epizootic were placed in the hot room for two hours, and the lice which soon came out to the extreme ends of the hairs were picked off, ground up with normal saline solution in a mortar and the emulsion filtered through a Berkefeld filter. The ground-up lice before filtering gave no growth of the bacillus in dulcite broth and the filtrate proved sterile on inoculation into broth. Animals were inoculated with the filtrate, the results being set forth in Table IX.

TABLE IX.



II. *Possible entry of the virus by feeding or nasal infection.*

Some experiments dealing with this point were carried out and have been already referred to.

III. *"Contact" experiments.*

It is a matter for some regret that especially during the earlier experiments when we had at our disposal a healthy stock of animals for experimental purposes contact experiments were not carried out. At this period the supply of guinea-pigs was somewhat restricted, and we preferred to use them for filtration experiments in order to discover whether or not the filtrates were pathogenic. We fully realise the importance of contact experiments in an investigation of this kind and should another opportunity arise in future we would endeavour to settle this point.

Occurrence of the Bacillus in Guinea-pigs apart from an Epizootic.

The bacillus was found in a high percentage of cases (vide supra) in the faeces and intestinal contents of guinea-pigs from the stocks affected by the epizootic between the second week in October and the first week in December. During this year the very large number of guinea-pigs under observation in connection with the routine examination of milk for tubercle bacilli have given the bacillus in only five instances in the heart-blood and organs, the intestinal contents not being examined as a rule. In May, two guinea-pigs out of four dying as the result of the injection with milk contained the bacillus in their intestines.

Six apparently healthy guinea-pigs were obtained in December from the totally unrelated stock at Elstree, where at the time no epizootic existed. Three of them were killed, and the organs were found to be quite healthy and gave no cultures. Of the other three one showed two small hard whitish yellow nodules in the spleen, which, however, gave no culture of the bacillus. The second showed a few white necrotic patches in the liver and four or five small whitish nodules in the spleen; a bacillus indistinguishable from that found in the present epizootic was cultivated from the spleen but not from the intestinal contents. The third showed a few yellow-white areas in the liver, and three or four yellowish white nodules in the spleen which gave a culture of the same bacillus.

At this time a few more deaths than usual were noted amongst the Elstree breeding-does but no definite epizootic existed. Dr MacConkey

kindly forwarded us two of these does; both of them showed numerous nodules in the liver and spleen and gave pure cultures of the bacillus.

It is of interest to note that similar appearances to these suggesting a chronic infection were frequently observed in guinea-pigs from the stock affected by the epizootic which died at various periods of some four to eight weeks after the height of the epizootic, and from many of these the bacillus was obtained in pure culture.

In December, the faeces of 15 guinea-pigs from a separate stock which remained unaffected by the epizootic were examined for the presence of the bacillus with negative results. Guinea-pigs from this stock were used for the earlier filtrate experiments, and as stated above gave cultures of the bacillus in 6 % of cases.

Between November and May the intestinal contents of 63 guinea-pigs used for inoculations in the ordinary routine examination of milk for tubercle bacilli were examined and the bacillus recovered in five instances, *i.e.* 8 %.

We may compare this with Uhlenhuth's finding of *Bacillus suipestifer* in the intestinal contents of 8 % of normal swine.

In nearly every case the strains of the bacillus referred to in this section were tested by agglutination as well as by cultural methods (see Table X.). It will be seen that all these strains corresponded in their agglutination reactions with the epizootic strain.

TABLE X.

Showing agglutination limits of various strains of the bacillus isolated from guinea-pigs apart from the epizootic. The serum used was obtained from a rabbit immunised with a strain of the bacillus isolated during the epizootic and agglutinated the homologous organism up to 1—10000.

Wright's method used—two hours at 37°, overnight at room temp.
The final reading is given.

	G.-pig inoculated with milk deposit. Killed 28 days later, T.B. found. Culture from small intestine	G.-pig inoculated with milk deposit. Killed 28 days later, T.B. not found. Culture from small intestine	G.-pig dead as result of inoculation of milk deposit. Culture from small intestine	G.-pig dead as result of inoculation of milk deposit. Culture from small intestine	Culture from spleen of apparently healthy Elstree g.-pig	Culture from spleen of apparently healthy Elstree g.-pig	Culture from spleen of Elstree breeding-doe	Culture from spleen of Elstree breeding-doe
100	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
1000	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
5000	+ + +	–	+ +	+ + +	+	–	+	–
10000	tr	–	–	–	–	–	·	–
Control	–	–	–	–	–	–	–	–

Discussion of Results.

The frequent finding of the bacillus in guinea-pigs dying during the epizootic as contrasted with its much less frequent occurrence in other guinea-pigs, at first sight strongly suggests that this bacillus was the causal agent of the epizootic.

Our results do not support this view in the following particulars:

1. It cannot be doubted that there was a spread of the bacillus from animal to animal during the epizootic as our culture results show, but the difficulty of killing guinea-pigs by feeding them with cultures of the bacillus almost negatives the idea that the epizootic was thus maintained and spread, and practically makes it necessary to postulate the presence of some other factor as the effective agent in the transmission of the disease. The migration of the bacilli from the intestines to the organs is probably merely a secondary invasion as in the analogous case of the *B. suipestifer* in swine fever.

2. This first objection is further strengthened by the results of our contact experiments in which contacts put with guinea-pigs infected either by feeding or subcutaneously and excreting the bacilli in the faeces rarely died, while the mortality in the epizootic was about 90 %.

3. Skin infection is the only other likely mode of transmission, and we have above given the evidence against this possibility.

4. We have evidence that the bacillus is found in guinea-pigs apart from an epizootic and in apparently healthy animals.

It is true, as is shown by one of us, that some of the survivors of the epizootic proved to be chronic carriers of the bacillus, that the survivors' serum definitely agglutinated the bacillus, and that they were immune to large doses of virulent cultures given subcutaneously. It must be noted, however, that in the course of a series of feeding experiments now in progress, we have found that guinea-pigs fed with cultures may excrete the bacillus in the faeces for some time subsequently while remaining apparently healthy and that the blood of some of these animals agglutinates the bacillus.

The general trend of the whole of these experiments leads us to the view that the bacillus is merely a secondary invader, and that alone it cannot explain the phenomena connected with the epizootic. We suggest that the primary factor in the transmission of the epizootic was a filter-passer and the high mortality amongst animals inoculated with sterile filtrates supports this view.

SUMMARY.

1. An epizootic killing 90 % of a stock of 500 guinea-pigs has been described; cultures from these guinea-pigs frequently gave an organism indistinguishable by cultural or serological tests from the *B. aertryck* and the *B. suipestifer*.

2. This organism was highly pathogenic when inoculated subcutaneously into guinea-pigs and of low pathogenicity when given to them with food.

3. Healthy contacts put with animals infected subcutaneously or fed with the bacillus did not die.

4. Sterile filtrates of organs of guinea-pigs of the infected stock administered by different methods frequently killed.

5. The evidence definitely suggests that the essential infecting agent in the epizootic was a filter-passer.

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