EPIDEMIOLOGY OF TYPHOID FEVER.

OBSERVATIONS ON THE SPONTANEOUS SEASONAL RECURRENCE OF PARATYPHOID EPIDEMICS AMONG GUINEA-PIGS.

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(With 2 Charts.)

I. The causative organism.

During the autumn of 1926 and again during the early summer and autumn of 1927, there were spontaneous epidemic outbreaks of a paratyphoid-like disease among the stock guinea-pigs. The causative organism of these epidemics was a bacillus having the general properties of the typhoid-paratyphoid group, but differing from any of the species heretofore described as responsible for such outbreaks among guinea-pigs. In the various epidemics previously described by Wherry (1908), Howell and Schultz (1922), Thomas (1924) and most recently Nelson and Smith (1927), the causative agent was a gas producing paratyphoid-like bacillus belonging to the general group of rodent paratyphi. The organism which was the cause of the local epidemics is distinctive in that it fails to produce gas from any of the sugars, and is serologically distinct from the other paratyphoid bacilli. This organism has been isolated repeatedly from three distinct epidemics all of which were extremely severe in character.

In view of the peculiarity of this organism and its etiological relation to the epidemics which have been studied in some detail, it is of interest to describe its characteristics and the general pathology of the disease produced by it.

The organism has the general characteristics of the typhoid group. It is an actively motile gram-negative bacillus. On agar and broth it produces a growth typical of the paratyphoid group of bacilli; transparent, opalescent colonies in the one, and a uniform turbidity in the other. It does not produce indol and does produce hydrogen sulphide. It ferments dextrose, maltose, mannite and xylose, and fails to ferment lactose, saccharose, dulcite, and arabinose. It does not produce gas in any of the sugars which it ferments. Serologically it is not agglutinated by typhoid or Gaertner anti-sera, but is agglutinated in a fairly high titre with *paratyphoid B.* serum (1 : 1600 to 1 : 3200, end titre of serum 8000). Absorption tests showed that the agglutination is non-specific; but it indicates an antigenic group relationship with *B. paratyphosus* B. Its relation to the members of the Salmonella group is indicated in Table I.

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Table I. *Fermentation reactions of typhoid-like cultures from guinea-pigs compared with those of other members of the typhoid-paratyphoid group.*

Group	Gas	Dextrose	Levulose	Arabinose	Maltose	Lactose	Saccharose	Mannite	Xylose	Dulcite	Raffinose	Indol	H_2S	Motility
B. X.	−	+	+	−	+	−	−	+	+	−	−	−	+	+
B. typhosus	−	+	+	−	+	−	−	+	+	−	−	−	+	+
B. paratyphosus B.	+	+	+	+	+	−	−	+	+	±	−	−	+	+
B. enteritidis	+	+	+	+	+	−	−	+	+	±	−	−	+	+
B. suipestifer	+	+	+	−	+	−	−	+	+	−	−	−	+	+
B. pestis caviae	+	+	+	+	+	−	−	+	+	−	−	−	+	+

The organism responsible for the epidemic seems to have distinctive characters. It belongs undoubtedly to the general group of *B. paratyphosus* B. But it is distinguished from the other species of the group by its constant inability to produce gas. In its fermentative reactions it is distinct from *B. pestis caviae* described by Wherry or the enteritidis type of rodent paratyphi; but resembles *B. suipestifer* very closely since, except for its failure to produce gas, it is identical with it.

Doctor Brown of the Wellcome Bureau, London, kindly compared the behaviour of this bacillus with that of members of the Salmonella group in relation to organic salts. His finding may be briefly summarised as follows: In respect to agglutination they appear to be related to the Salmonella group but not so when tested by the absorption test. They fail to decompose trisodium citrate, and sodium mesotartrate, but decompose sodium dextro- and laevo-tartrate, sodium mucate and fumarate. No other organism in his experience has this reaction on the organic salts, and it is most unusual for an organism to decompose sodium tartrate and to have no action upon sodium citrate.

The fact that the identical organism has been isolated in three different epidemics, the constancy with which it retains its distinctive cultural and fermentative characters, and its specific virulence for guinea-pigs would indicate that we are dealing with a distinct species responsible for the epidemics among our guinea-pigs in Jerusalem.

Pathologically the disease produced by this organism resembles the usual picture of paratyphoid infections in guinea-pigs. The changes and the degree of involvement of the different organs are not uniform, but depend on the age of the animals and the duration of the disease. Young guinea-pigs may succumb within a week after exposure with manifestations of septicaemia, with abundant fibrinous exudate in the peritoneum and general hyperaemia. In older and more resistant animals the disease usually lasts longer and in them are found various gradations of lesions including the characteristic picture of pseudotuberculosis with numerous abscesses in the liver and spleen. One of the most constant changes is that of the gall bladder. In most of the cases the gall bladder was distended and filled with a white purulent fluid containing large masses of the bacilli. In the first epidemic diarrhoea was a constant symptom; this was not a constant feature of the other epidemics.

The following is a composite picture of the gross lesions usually encountered: general hyperaemia of the abdominal wall and viscera. The glands are enlarged and hyperaemic. As a rule there is a greater or lesser amount of serous exudate, sometimes fibrinous, sometimes clear and haemorrhagic. The stomach is usually empty; the small intestine congested and often inflamed. In case of longer duration the Peyer's patches are prominent, inflamed and oedematous; in acute cases the changes are not so noticeable macroscopically. The liver is enlarged, dark and more or less friable; in more chronic cases it may have many large and small abscesses full of bacilli. The spleen is always enlarged, dark and extremely friable; in the great majority of cases the spleen has a mottled appearance as a result of the numerous abscesses. Cholecystitis is a typical lesion; the gall bladder is nearly always distended and filled with a greyish pus rich in bacilli. The wall of the bladder is thickened. The kidneys are hyperaemic and the suprarenals congested and haemorrhagic.

Source and virulence of cultures. Cultures were always made from the heart-blood, spleen, liver and gall bladder contents. The primary cultures were either made in glucose broth or on MacConkey plates. Heart-blood cultures were made in dextrose broth. The liver, spleen and gall bladder cultures were made directly on MacConkey plates. From these cultures transplantations were made to lead-acetate triple sugar, and characteristic cultures were agglutinated with a specific antiserum made with the original strain isolated during the first epidemic. Over one hundred cultures isolated during the various epidemics proved identical both culturally and serologically.

The organism is highly virulent for guinea-pigs. Relatively small doses (50,000,000 organisms) fed to a guinea-pig weighing 350–400 grm. produce a typical infection with fatal termination in two weeks. Younger animals succumb in a much shorter time. The lesions in the experimental infections do not differ essentially from those seen in the spontaneous infections.

The organism has also been recovered from two white rats, maintained on defective diets and kept in the same room as the guinea-pigs, and from one rabbit. It does not, however, seem to be virulent for these animals or tend to cause epidemics among them. A large number of white rats and a considerable number of rabbits were kept in the same room with the guinea-pigs during the first epidemic with only two fatalities among the rats and one among the rabbits. Half an agar slant fed to white rats weighing 40–50 grm. failed to produce any outward signs of infection, and the organisms promptly disappeared from the faeces.

The organism is extremely toxic. Small doses of dead bacilli injected intravenously into rabbits for purposes of immunisation caused the death of the animal after three or four injections. This occurred despite the rapid production of antibodies. In experimentally or spontaneously infected guinea-pigs the intraperitoneal or subcutaneous injection of killed vaccine hastened the death of the animal instead of aborting the disease. This organism appears to be more toxic than any of the other members of the Salmonella group.

II. THE SEASONAL RECURRENCE OF EPIDEMICS.

The experimentally controlled studies of the epidemiology of mouse typhoid by Flexner (1922), Amoss (1922), Topley (1919) and Webster (1924), have shed light on the epidemiology of enteric infections. Of particular significance is the evidence presented by Webster regarding the importance of dosage and host susceptibility in the spread of these epidemics.

There are, however, a number of elements in the epidemiology of these diseases which remain obscure. Outstanding among these is the periodic alternation of endemicity and epidemicity, and more particularly the seasonal prevalence of epidemics. It was hoped that the observations of the spontaneous outbreaks of the epidemics in the guinea-pigs might shed some light on this phase of the problem. The first outbreak occurred in October 1926 and the second and third in July and October, respectively, of 1927.

History of the epidemics. Towards the middle of July 1926, eighty guinea-pigs were brought from Egypt. But for an occasional death from unascertained causes, nothing unusual occurred. It was especially noted that no diarrhoea occurred among the animals.

On August 13th, twenty-four additional guinea-pigs were brought from Ramalla, Palestine, several of them being pregnant.

On August 20th, one of the Ramalla pigs gave birth to two animals. After ten days these young both died with symptoms of diarrhoea and paratyphoid, but no cultures were made.

On August 23rd and August 25th, five more guinea-pigs were born to Ramalla sows, which also died subsequently with similar symptoms.

On September 9th and September 10th, two of the adult guinea-pigs from Ramalla died, but the cause was not ascertained.

On September 21st, one of the Egyptian guinea-pigs died with definite symptoms of diarrhoea and paratyphoid, and on September 27th the epidemic started in earnest.

Between September 1st to September 21st, seven new-born and three adults died.

From September 27th to October 3rd, seventeen guinea-pigs died.

From October 4th to October 10th, seven guinea-pigs died.

From October 11th to November 24th, nine guinea-pigs died.

The outbreak had all the manifestations of an acute epidemic; the early scattered cases, the sudden flare up and the gradual subsidence with continuation of sporadic cases. The epidemic started among the newly introduced pregnant Ramalla pigs, and after a month the Egyptian animals were attacked. Both groups of animals suffered equally in the end. Altogether 9 Ramalla and 31 Egyptian animals succumbed to the infection, or 37·5 per cent. of the former and 38·7 per cent. of the latter; there being practically the same mortality in the two groups.

These were the first animals introduced into the animal house. They were

kept in small groups of four or five in cages arranged on shelves around the wall in a room measuring 7 × 4 metres. In the same room were kept between 50–100 white rats of all ages and 20 to 25 rabbits; but only the guinea-pigs were affected.

On November 25th, a new lot of 60 guinea-pigs, averaging 250–300 grm. in weight, were brought from Egypt. These animals were purposely placed in cages together with some of the survivors of the epidemic and kept in a separate room. On November 27th, one, and on December 6th, another of the surviving animals succumbed to paratyphoid. On December 6th, the first animal of the new lot appeared sick. On the 7th, another of the old guinea-pigs, and on the 8th, two of the new lot died. No further deaths occurred subsequently.

During the entire winter and spring, not a single guinea-pig died from the paratyphoid infection. The animals were used for various experimental purposes, and every animal was subsequently autopsied and examined for signs of paratyphoid infection, but neither the spleen nor the liver showed any gross indication of infection, and routine cultures made from the heart-blood were negative. Only in one case was a positive culture obtained from the spleen.

No new guinea-pigs were imported during the winter and spring. The animals were bred on the spot and it appeared that the infection had disappeared.

On July 23rd, 1927, the first fatality from the paratyphoid occurred in our stock—with the death of an old gravid female weighing about 500 grm. There followed rapidly the death of two animals on the 25th, two on the 26th and three on the 28th. All of these were young animals born in the animal house and ranging between 200 and 300 grm. in weight. This marked the beginning of the second epidemic. The weekly distribution of deaths is shown in Table II.

Table II. *Weekly number of deaths during the second epidemic—July* 1927.

Weeks	23. vii.–29. vii.	30. vii.–5. viii.	6. viii.–13. viii.	14. viii–20. viii.	21. viii.–26. viii.
No. of deaths	9	12	20	0	0

The epidemic lapsed suddenly owing to exhaustion of the susceptible stock. On August 19th, ten young guinea-pigs, recently brought from Egypt for another laboratory, were added to the infected boxes, and a week later another six. On the 27th, two more of the old animals died and on September 2nd and 4th, one each of the new animals died. Then again there were no deaths until the 16th. During the week of the 16th–22nd, five of the recently imported animals died. From the rapid succession of deaths it appeared that a new wave had begun, but this stopped short because of the lack of susceptible animals, since our stock of untreated animals amounted now to only 15–20.

There are a number of interesting points in connection with the July epidemic. The first death occurred 12 days after an earthquake which resulted in a general disturbance including neglect in the care of the animals. The first

animal to succumb was an old gravid female, while the ones immediately following were young animals. The smaller animals died during the early part of the epidemic, while the larger ones were attacked much later. The sequence of deaths according to weight is shown in Table III. Evidently there was a greater primary resistance manifested by the older animals, although in the end they succumbed in as large a proportion as the younger ones.

Table III. *Sequence of deaths of young and old animals.*

	Weights in grammes			
Week	Up to 200	200–300	300–400	400 up
23. vii.–29. vii.	2	5	3	1
30. vii.–5. viii.	4	5	2	1
6. viii.–13. viii.	2	1	3	14
Totals of deaths	8	11	8	16

There were three questions of interest in connection with these two epidemics: (1) the source of the organism, (2) the relative virulence of the strains obtained from the two epidemics, and (3) the reason for the sudden flare up of the disease after a quiescence of seven months.

During the first epidemic it was apparent that the organism had been introduced by the guinea-pigs brought from Ramalla and that the outbreak was started with the death of the new-born animals. The guinea-pigs brought from Egypt were apparently healthy when they arrived in July and were not in contact with any other guinea-pigs. The Government Laboratories receiving their animals from the same source in Egypt experienced no epidemic. The possibility of the white rats being the carriers was also excluded through the evidence afforded by the cultures of faeces. Similarly cultures made from the spleen and faeces of a dozen wild rats caught in and around our animal house were negative for the organism.

It is difficult to attribute the second epidemic to an external agency. The guinea-pigs were bred in the animal house and no new guinea-pigs had been introduced. The most plausible explanation is that the surviving guinea-pigs remained carriers. That the number of these carriers must have been rather small is indicated by the fact that only a single positive culture was obtained in some thirty autopsies made during the winter and spring months. Even during the epidemic the number of bacilli excreted by the guinea-pigs was very small. In general, guinea-pig faeces contain only few bacteria. Repeated examinations made of the faeces from the guinea-pig boxes during and after the epidemic yielded the characteristic epidemic strain only intermittently and in small numbers. The presence of many bacilli was followed within two or three days by the death of one or more animals, indicating that more active excretion of bacilli occurs shortly before death.

The following procedure was followed in the examination of faeces: one gramme of faeces was emulsified in 10 c.c. of saline and 0·05 c.c. spread over a brilliant-green and MacConkey plate. Since the number of bacteria in

guinea-pig faeces is relatively small, fairly large amounts of faeces emulsion could be spread out on the plates. After incubating for 24 and 48 hours, typical colonies were inoculated into lead acetate double sugar medium. Characteristic cultures were agglutinated with specific sera.

The results of the examinations are recorded in Table IV.

Table IV. *Presence of the epidemic strain in the animal cages.*

Date	No. of boxes examined	No. of boxes positive
1. viii.	16	0
4. viii.	19	0
12. viii.	9	1
16. viii.	9	1
18. viii.	9	2
25. viii.	4	0
30. viii.	11	0
7. ix.	12	1

Tests showed that the strains isolated during or after the epidemic did not differ appreciably in virulence from those obtained from carriers or from animals which died during this year's epidemic. Feeding 100,000,000–200,000,000 organisms caused death with typical symptoms after about two weeks. Apparently the spontaneous infection occurred by the repeated ingestion of small doses and the infection progressed more or less rapidly according to the resistance of the host and the number of bacilli ingested.

But, even granting that to have been the case, it is difficult to account for the absolute quiet during the winter and spring months and the sudden flare up in July. The number of susceptible animals was as large in November, April, or May as in July.

In this connection the third epidemic is of interest. This epidemic occurred in the laboratory of the Hospital. Towards the end of July about 250 guinea-pigs were brought from Egypt. These were apparently healthy. Vaccination against the paratyphoid strain was carried out on twenty-five of these animals without any losses, whereas vaccine treatment of infected animals was found to excite a more rapid evolution of the infection.

About two weeks before the new animals were brought, two guinea-pigs had been borrowed from the University Laboratory. These had received vaccine by mouth and were apparently healthy. Moreover they were kept apart from the new Egyptian stock.

During August, a number of animals died, but no study was made of them. Beginning with September, however, every dead guinea-pig was autopsied and cultures made from the heart-blood, spleen and gall bladder. During September, 11 animals died of infection with the epidemic strain. In October, 67 animals died. In November, 12. In the first half of December, 3. The percentage mortality in relation to the living population was as follows: September, 5·5; October, 49; November, 26; December, 10.

These results are a repetition of the September-October epidemic of 1926. The epidemic was more severe in character as a result perhaps of the larger number of young animals, greater crowding and slighter precautions in the

handling, cleaning and feeding of the animals. The difference between the two epidemics is that in 1926 the infected stock was introduced into a healthy population, while in 1927 the reverse occurred; the healthy population was introduced into an infected group.

There remains to be explained, however, the striking difference in the effect of the introduction of a healthy population into an infected group at the end of November 1926, as compared with that in July. The new animals brought at the end of November 1926 were exposed to much the greater risk, being in close contact with sick animals as well as the survivors of a recent epidemic; yet they hardly suffered at all. On the other hand those brought in 1927 and subjected to a less risk of infection, suffered from a severe epidemic.

This is as difficult to explain as is the sudden outbreak in July 1927.

DISCUSSION.

It is of interest to compare the epidemic trend in the guinea-pig population with that of typhoid fever in a human population. The epidemic curve of typhoid fever in Palestine is binodal with one peak in July and the other in October. During the hottest month (August), there is a drop in infections, while during the months of January to May inclusive, the infection rate is minimal. This trend is in striking correspondence with the course of events in the guinea-pig population. There is a definite seasonal recurrence in both, for equally obscure reasons.

There are two points of interest in connection with the reported epidemics: (*a*) these epidemics indicate clearly that the introduction of an infected population into a healthy community has the same effect as the bringing of a healthy susceptible population in contact with an infected one; (*b*) the importance of season in relation to the epidemics. The introduction of a new population into an infected one in November, was not followed by any bad results until the following July, while such an immigration at the end of July resulted in an epidemic in October. It should perhaps be added that the animals were kept in a temporary shed more or less under natural conditions of temperature and humidity, except during the severe winter months—January and February —when artificial heat was employed.

As in the human epidemics the question is: why the outbreaks occurred at a given fixed season? The susceptible material was as abundant in April and June as in July and September. The cultures seemed to have a constant level of virulence. The only likely explanation is that the animal organism undergoes a profound seasonal modification in susceptibility. The observation by Arnold and Brody (1926, 1927), that the reduced acid excretion in the stomach at high external temperatures renders infection easier, does not account for the reduction of enteric infections during August—the hottest month of the year. In experimental infections in animals, infection occurs readily at a room temperature of 24° C. It would seem that in the case of enteric infection there must be, in addition, some local change either in the intestinal mucosa or in other

organs, rendering animals more vulnerable to the localisation of the infecting agent. The relation of season to organ changes has been pointed out by Brown, Pearce and Van Allen (1924, 1925). Similar changes may also occur in tissues rendering them more or less vulnerable to invading organisms. Work done in this laboratory during the last two years[1] indicates that there is a seasonal

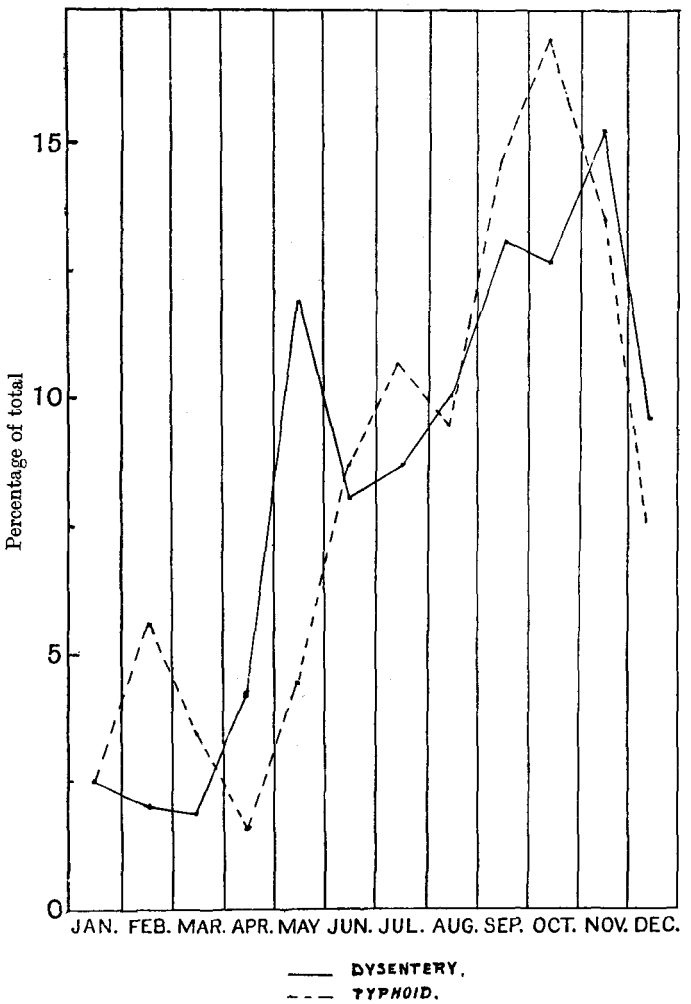

Chart 1. Prevalence of typhoid and dysentery during the year; mean of five years. (Percentage of total positive cases.)

variation in the faecal bacterial flora as well as in the intestinal flagellates of normal individuals. The changes in the flora appear about April or May—the time when the epidemics of bacillary dysentery begin. It would seem that there are seasonal changes in the environment or substrate of the human

[1] Kligler and Goldwasser, unpublished.

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intestine, which render it more or less suitable for the development of one or another group of organisms.

The significance of this factor of increased seasonal susceptibility is emphasised by the epidemiology of other diseases. Dysentery, for example, has a seasonal prevalence in Palestine distinct from that of typhoid. The first dysentery peak occurs with great regularity in the month of May, whereas the first typhoid peak occurs in July. Yet both are intestinal infections differing only in their localisation. Arnold's experiments, though highly suggestive, fail to account for this difference in two enteric infections. Similarly in another type of disease, malaria, there is, even in a native untreated population, a totally different seasonal prevalence of the benign (*Plasmodium vivax*) and malignant (*P. falciparum*) forms. Charts 1 and 2 are illustrative.

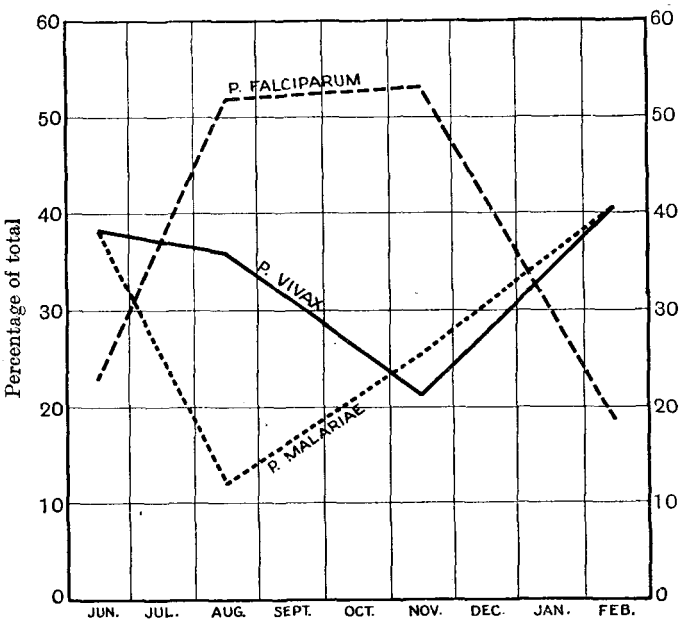

Chart 2. Prevalence of different forms of parasites in various seasons. (Percentage of total positive parasites.)

It would seem, therefore, that for the development of a sound theory of the epidemicity of certain diseases it is necessary to introduce another factor. To the factor of virulence stressed by Topley and of dosage so clearly established by Webster, must be added that of seasonal variation in the physiological condition of the host which renders it possible for a smaller number of organisms of a given level of virulence to establish themselves in the host, multiply and start an epidemic. In other words, at the proper season the critical dose or the level of virulence need not be so high to produce an infection as at other periods of the year.

The nature of the changes which occur in the host is a problem for future investigation. The work of Arnold and Brody, Brown, Pearce and Van Allen,

and the effects of radiation on resistance reported by Kligler and Weitzman (1926) are suggestive and indicate the direction of such investigations.

Summary.

A series of spontaneous epidemics of a paratyphoid infection among guinea-pigs is described. It is shown that an epidemic may result from the introduction of a fresh susceptible population into an infected area and *vice versa*. Such epidemics developed only when the new population was exposed during certain months. The bringing in of a fresh population at the end of November was not followed at the time by an epidemic outbreak; the epidemic did not occur until the following July.

The observed seasonal occurrence was not due to a change in virulence. The strains isolated at the end of the 1926 epidemic did not differ in virulence from those cultivated at the height of the 1927 epidemic.

The guinea-pig may remain a carrier for a long time whilst eliminating bacteria in the faecal discharges only intermittently and in small numbers. In general, even in the case of infected animals, the discharge of pathogenic bacteria is relatively small, except a few days before death.

Attention is called to the parallelism between the seasonal prevalence of human typhoid epidemics in this country and the epidemics of paratyphoid observed in our guinea-pig stock.

It is pointed out that a sound theory of the epidemiology of certain diseases must take into account the seasonal variation in host susceptibility.

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