GAERTNER GROUP BACILLI IN RATS AND MICE.

By WILLIAM G. SAVAGE, M.D., B.Sc., D.P.H., and W. J. READ, M.Sc., F.I.C.

Our knowledge of the importance of the Gaertner group of bacilli in human and animal pathology has been slowly but steadily extending. Members of this group are etiologically associated with most of the cases of paratyphoid fever while the number of outbreaks of food poisoning shown to be due to these bacilli is rapidly increasing. In addition they have been found to be associated with a number of diseases in the lower animals.

The group may be divided into two sub-groups, one forming the true Gaertner organisms including *B. enteritidis*, *B. suipestifer*, *B. paratyphosus* β and possibly a few other strains, and the other consisting of closely allied but apparently non-pathogenic bacilli found in the healthy intestine (para-Gaertner bacilli).

The distribution in nature of this group of organisms is obviously of considerable importance and has been extensively studied during the last few years although previously almost entirely neglected. For a summary of the available data as to the distribution of these bacilli amongst man, animals, food products and in nature the Report by one of us to the Local Government Board (Savage 1913) may be consulted.

Briefly stated it may be said that when the closely allied para-Gaertner bacilli are differentiated, true Gaertner group bacilli are not present, or only found extremely rarely, in the healthy human intestine and with nearly equal rarity (apart from rats and mice) in the healthy animal intestine. Put another way, and excluding for the moment rats and mice, the available English data certainly negatives the view that these organisms are natural intestinal inhabitants of man or animals although such a distribution is frequently asserted to exist.

On the other hand while the evidence is against the view that true Gaertner bacilli are natural inhabitants of the healthy alimentary tract

of man and the domestic animals used for food it is now well known that this group of organisms is responsible for a good deal of disease in animals. Of such diseases may be mentioned some forms of septicaemia and other diseases of calves, pyaemic and septicaemic conditions in cows and other bovines, some cases of enteritis in cows, disease in parrots (psittacosis) and sparrows, and disease in rats and mice.

While it may be advanced with some confidence that the presence of members of the true Gaertner group in the domestic animals used for food is evidence of some relationship to a diseased condition or association with cases of disease due to these bacilli ("carriers") the evidence is not very definite as regards rats and mice. Some of the available evidence suggests that in these animals Gaertner group bacilli may be natural intestinal inhabitants and present as such, apart from any association, direct or indirect, with a diseased condition. Indeed Bainbridge (1912) in the Milroy Lectures for 1912 on paratyphoid fever and meat poisoning suggests, "It may well be that the rat's intestine is the true home of this organism (*i.e. B. enteritidis*), and that it reaches the alimentary canal of cattle and other domestic animals through the contamination of their food or bedding by rats."

As will be shown later the matter is not merely of scientific interest but is of real practical importance in relation to food poisoning outbreaks and other diseases due to this group of organisms.

There are a number of investigations which bear upon this question.

Uhlenhuth and Shern (quoted by Hübener 1910) frequently found Gaertner bacilli in the spleen of healthy tame rats and observed that rats inoculated intraperitoneally with rat serum often died from an outbreak of Gaertner enteritis.

Heuser (1910) examined about 100 mice and in 5 per cent. isolated Gaertner bacilli of both types (*i.e. B. enteritidis* and *B. paratyphosus vel B. suipestifer* according to German classification) from their intestinal contents. The mice showed no symptoms of disease. He also examined about 60 white rats and found *B. enteritidis* in about 5 per cent. but no hog-cholera bacilli (*B. suipestifer*). He found that by animal passage the bacilli of the hog-cholera group showed a distinct rise of virulence to rats.

Zwick and Weichel (1911) examined 177 mice and in 28 found Gaertner group bacilli.

Eckert (1909) examined a large number of samples of intestinal contents including those of five rats. He failed to find any Gaertner group bacilli in the rat excreta.

Evidence is also available in another direction. A number of observers have fed or infected mice and rats with material of different kinds and from these animals in a number of the cases Gaertner group bacilli have been recovered post-mortem. The material injected in many of the cases certainly did not contain Gaertner group bacilli as shown by its nature and from careful cultural investigations. The following may be mentioned.

Mühlens, Dahm and Fürst (1908) fed mice with different kinds of prepared food and a number of them died, Gaertner group bacilli being isolated. In all 74 died out of the 138 mice fed and from nearly all of these one or other member of the Gaertner group was isolated. They were all highly virulent. These bacilli could not be found in any of the foods by cultural examination.

Zwick and Weichel (1911) fed 140 white mice with 70 samples of salt meat and different forms of pig, all of which culturally examined failed to show Gaertner group bacilli. 85 (60·7 per cent.) of the mice died and from two of them *B. paratyphosus* β was isolated.

Somewhat similar results have been found by other observers. While these findings have usually occurred with rats and mice comparable results have been met with in guinea-pigs. For example Smallman (1905) injected over 200 guinea-pigs intraperitoneally or subcutaneously with cultures of *B. typhosus* either living or dead. In 22 instances (about 11 per cent.) organisms of the Gaertner group were obtained.

With these outbreaks must be associated the fact that spontaneous outbreaks of infectious disease amongst rats and mice are not uncommon. One of us has met with three definite outbreaks in his laboratory mice at widely separated intervals, two due to or associated with *B. enteritidis* and the third also due to a Gaertner group organism, the precise sub-group not being investigated.

The above facts suggest two separate possibilities. They may be taken as showing that Gaertner group bacilli are natural inhabitants of rats and mice, or they may be explained on the view that they are not really natural inhabitants, but when found are present either as "carriers" from contact with actual cases or are present after recovery from an infection with Gaertner bacilli not severe enough to be fatal.

The latter view seemed to us to be the more probable but to try and clear up the matter we have carried out an extended series of examinations of rats in this country.

Summary of investigations.

The work carried out consisted of the bacteriological examination of 41 rats obtained from different sources. All the rats were of the ordinary brown variety except two of the Cardiff animals, obtained from a ship from India, which were black rats.

Twenty-eight of the rats were obtained in Weston-super-Mare mostly killed on the refuse heaps of the town, the remaining few being killed in houses.

Ten of the rats were obtained from Cardiff through the kindness of Dr Walford, M.O.H. Seven of these were caught on board ships coming into Cardiff Dock, three were from houses in the city. The remaining three rats were from a small town in Somerset about 30 miles from Weston-super-Mare.

Methods of examination.

All the rats were as far as possible examined within a few hours of being received.

The same method of examination was followed throughout. The naked eye appearances of the organs were noted in each case.

Cultivations were made in the ordinary way from the spleen, liver and heart blood. The cultivations from the spleen and liver were into malachite green dulcite broth, that from the heart blood into ordinary nutrient broth. If growth took place the liquid culture medium was plated upon neutral red lactose bile salt agar (L.B.A.) and the colonies identified. If there was no growth after two days at 37° C. the cultures were regarded as sterile.

In addition to these organs the intestinal contents were also examined. The examination was both direct and by enrichment. For the direct examination scrapings from both large and small intestine were added to sterile water in a test tube and an emulsion made. This emulsion was used to brush four L.B.A. plates in the ordinary way. After incubation for 24 hours at 37° C. the white colonies were picked off and subcultivated.

For the enrichment method loopfuls of intestine scrapings were added to tubes of dulcite malachite green broth and incubated, usually for 24 hours, occasionally for 48 hours. If as was usually the case growth resulted this was plated on several L.B.A. plates and the white colonies investigated as for the direct plates.

The subcultivation method adopted for sorting out the possible true Gaertner group bacilli from the pseudo or para-Gaertner forms and non-Gaertner bacilli was to inoculate the white colonies into a compound sugar broth (C.S.B.). This was ordinary nutrient broth in double tubes containing 0·3 per cent. each of lactose, saccharose and salicin. True Gaertner bacilli do not ferment any of these substances so that if gas is produced the culture could be at once discarded as not a true Gaertner organism.

If after two days no gas production occurred the culture was subcultivated into glucose broth, litmus milk, peptone water, dulcite broth and upon gelatine slope. All organisms which reacted to these media like true Gaertner bacilli were then very fully worked out after being replated. Agglutination and pathogenicity tests were also employed.

The dulcite malachite green broth was used as an enrichment medium as in our experience it exerts, in the proportions used, a restraining action upon *B. coli* group organisms while encouraging Gaertner group bacilli to grow.

It may be added that the methods employed were all of proved usefulness and were methods which had been used very extensively by one of us (Savage, 1907–1910) for similar investigations of other materials.

For a number of the rats, in addition to cultural examinations, samples of blood from the heart were collected and serologically examined. The results are recorded below.

Results obtained.

It will serve no useful purpose to give the details of each individual bacteriological examination.

Rats obtained outside Weston-super-Mare. There were 13 rats in this group. No true Gaertner group bacilli could be isolated from any of them either from the liver, spleen or heart blood or from the intestinal contents. In a few instances pseudo-Gaertner strains were isolated.

Rats obtained from Weston-super-Mare. There were 28 in all in this group and somewhat different results were obtained. In 23 of these rats true Gaertner group bacilli could not be isolated either from the internal organs, heart blood or intestinal contents. From the remaining five rats true Gaertner group bacilli were isolated. The bacteriological findings in these five rats were as follows:—

Rat, No. 4. Examined April 24th, 1912. Obtained from town refuse heap. Numerous whitish pin-point areas on liver and scattered through the spleen.

Gaertner group bacilli isolated from spleen and liver (R_1 and R_2 respectively). Heart blood culture showed no growth. Although both the primary and secondary plates from the intestine showed white colonies none of these were Gaertner group bacilli.

Rat, No. 6. Examined April 24th, 1912. Same source as No. 4. One small apparently necrotic area on the liver, otherwise no naked eye abnormality. A Gaertner group bacillus isolated from the spleen (R_3). Cultures from heart blood and liver showed no growth. Numerous white colonies on both primary and secondary plates from the intestinal contents, but since all fermented the compound sugar medium or decomposed milk no Gaertner group bacilli were found.

Rat, No. 7. Examined April 25th, 1912. Same source as No. 4. No naked eye pathological appearances found. A Gaertner group bacillus (R_4) isolated from the spleen. Cultures from heart blood and liver showed no growth. The primary and secondary plates from the intestinal contents showed only a moderate number of white colonies. None of these were true Gaertner group bacilli but pseudo-Gaertner bacilli were fairly numerous.

Rat, No. 12. Examined May 20th, 1912. Obtained from a house. Rat had apparently died a day or so previously as organs were foul smelling. Apart from obviously putrefactive changes no abnormalities noticeable. Gaertner group bacilli isolated from the spleen and liver (R_5 and R_6 respectively). The heart blood culture showed growth but no Gaertner group bacilli were present as all slowly fermented the compound sugar medium. The plates from the intestinal contents both primary and secondary were all negative as regards the presence of true Gaertner group bacilli although pseudo-Gaertner bacilli were isolated

Rat, No. 13. Examined May 20th, 1912. Found dying in a house and killed. No abnormalities noted. True Gaertner group bacillus (R_7) isolated from the spleen. Cultures from the liver and heart blood showed no growth. No Gaertner group bacilli could be isolated from the intestinal contents.

Summarising all the results it will be noted that true Gaertner bacilli were not isolated in any case from the heart blood or intestinal contents, but that in five rats true Gaertner group bacilli were isolated, from the spleen in each case, in two of these from the liver in addition.

The true Gaertner group bacilli isolated were culturally worked out and showed all the usual cultural characters including fermentation of glucose and dulcite, absence of fermentation of lactose, saccharose, salicin and glycerine, no indol production and acid followed by alkaline production in milk. They were all actively motile bacilli.

Their position in the Gaertner group (except the organisms isolated from rats 12 and 13), was determined by a series of agglutination tests. The Gaertner bacilli from rats 12 and 13 were obviously obtained from rats infected and ill from the Danysz rat virus which had been used and may be assumed to be that strain. The agglutination reactions of the others are shown in the following table.

Serum and dilution		R_1	R_2	R_3	R_4	*B. aertrycke*	*B. enteritidis*	*B. paratyphosus β*
B. enteritidis serum	1:100	+	+	+	+		+	
	1:1000	+	+	+	+		+	
	1:3000	+	+	+	+		+	
	1:5000	+*p.*	+*p.*	+*p.*	+*p.*		+*p.*	
B. aertrycke serum	1:100	–	–	–	–	+		
	1:1000	–	–	–	–	+		
	1:2000					+		
	1:4000					+*p.*		
B. paratyphosus β serum	1:100	+*p.*	–	–	–			+
	1:1000	–	–	–	–			+
	1:4000	–	–	–	–			+

All reactions microscopic: time two hours. +*p.* = partial reaction.

The above table shows clearly that the four tested strains are identical with one another and are all identical with *B. enteritidis.*

The pathogenicity of the three strains R_1, R_3, R_4 was tested. All three were highly virulent to guinea pigs both on subcutaneous and intraperitoneal injection. Death in the latter case within 24 hours. Organisms recovered in pure culture from the spleen and other internal organs and completely identified with the injected bacilli.

In addition to the above bacilli culturally identical with *B. enteritidis* and other true Gaertner organisms a number of pseudo-Gaertner or para-Gaertner bacilli were met with. This name has been given by one of us to a group of organisms which culturally closely resemble true Gaertner group bacilli and which can only be culturally distinguished when an extended series of tests is employed, such as the fermentation of dulcite, salicin and glycerine. They were not specially looked for in the present investigation and indeed by the use of the compound sugar test all the salicin pseudo-forms were cut out and could not be identified. The chief pseudo-Gaertner forms found were therefore of the dulcite negative type, *i.e.* they were culturally identical with the true Gaertner strains except that they failed to ferment dulcite. Such organisms were isolated from the intestinal contents of rats 7, 9, 12, 14, 18, 19, 26, 28 and 37. Perhaps of greater interest is the fact that these dulcite negative strains were isolated in two cases from the internal organs, *i.e.* from the spleen of rat 19 and the heart blood of rat 25.

The chief interest attaching to these organisms is the fact that unless an extended series of cultural tests with these sugar-alcohol bodies is carried out they may easily be mistaken for true Gaertner organisms.

A further point of interest is the extent to which the sera of the rats react to Gaertner group organisms as evidence of old infection with

members of this group. Unfortunately we did not consider the importance of this point in our earlier cases and serum from only seven rats was collected.

These sera specimens were tested with all three varieties of the Gaertner group and also specially with Danysz's bacillus which is indistinguishable from and evidently identical with *B. enteritidis*. Tested in dilutions of 1 : 50 (time 1½ hours, microscopic method) the sera of rats 36, 38 and 40 failed to agglutinate any of the four strains. The other four showed some agglutinative properties as follows.

Rat serum	Dilution	*B. suipestifer*	*B. paratyphosus β*	*B. enteritidis*	Danysz bacillus
Rat 27	1 : 50	+	+	+	+
,,	1 : 100	+	+	+	+
,,	1 : 500	+	+	+	+
,,	1 : 1000	+*p.*	+	+	+
,,	1 : 2000	–	–	+*p.*	+
,,	1 : 5000	–	–	–	–
Rat 37	1 : 50	+	+	–	–
,,	1 : 200	+*p.*	+		
,,	1 : 500	–	–		
Rat 39	1 : 50	–	–	+	+
,,	1 : 100	–	–	–	*tr.*
,,	1 : 200	–	–	–	–
Rat 41	1 : 50	+	+	+	+
,,	1 : 100	+	+	–	–
,,	1 : 500	+	+	–	–
,,	1 : 1000	–	–	–	–

All reactions microscopic: time 1½ to 2 hours.
+*p.* = partial positive reaction. *tr.* = traces of reaction.

We do not think any significance can be attached to the slight reactions of the sera of rat 39 or possibly of rat 37 but the reactions obtained with rats 27 and 41 certainly point to an old infection with Gaertner group bacilli. Both these rats were obtained from the old refuse tips at Weston-super-Mare. From none of these rats were Gaertner group bacilli isolated.

Conclusions.

The results show that Gaertner group bacilli were only isolated from rats obtained in Weston-super-Mare. Inquiry showed that in November 1909, about 2½ years before the first rats were examined, the refuse tips and slaughter houses had been extensively dosed with the Danysz virus. None had been used subsequently until May 1912.

On May 8th, 1912, twelve days before rats 12 and 13 were examined, twelve tubes of this virus were distributed in the slaughter houses while further tubes were used in other parts of the town. The Gaertner bacilli isolated from these rats which were found dying in the treated houses were obviously due to direct infection. The bacilli isolated from the other rats were all *B. enteritidis*, while Danysz virus is a true *B. enteritidis*.

It is difficult to resist the conclusion that these bacilli isolated from the rats were from an old—possibly years old—infection with this virus. If this be accepted they are evidence of an old infection and lend no support to the view that Gaertner group bacilli are natural inhabitants of the rat's alimentary canal. The completely negative results as regards finding these bacilli in the intestinal contents strongly support this view.

The persistence of bacilli in carrier cases has been noted by several observers. Thus Petrie and O'Brien (1910) succeeded in experimentally producing the carrier state in guinea pigs by feeding them with *B. suipestifer*. In the case of two animals (out of six fed) the bacilli were isolated from the faeces for as long as 59 days after the last feeding. In an epizootic amongst the stock guinea pigs at the Lister Institute due to *B. suipestifer* recorded by O'Brien (1910) the survivors showed definite immunity to this bacillus, and five of them proved to be carriers, excreting the bacillus intermittently five months later.

It is well known that rats fed with Danysz's bacillus often recover, and are then highly immune and can eat large amounts of virulent virus without apparent ill health. The ingested bacilli are present in large numbers in the excreta.

The few sera of rats tested are further evidence of old infection in certain cases.

Although no Gaertner group bacilli were isolated from the intestinal contents it is probable that for some time after infection these bacilli are excreted in the faeces. Rats frequently infest slaughter houses and there is considerable likelihood of the meat being infected with their excreta. That such excreta may contain actively virulent bacilli quite identical with *B. enteritidis*, the cause of so many outbreaks of food poisoning, cannot be considered satisfactory.

It is of interest to note that the Gaertner group bacilli isolated were highly virulent.

SUMMARY.

Forty-one rats examined—internal organs and intestinal contents—for presence of Gaertner group bacilli. Bacilli identical with *B. enteritidis* isolated from five rats in each case from the spleen (in two from liver also). No members of this group isolated from the intestinal contents.

Several of the rat sera were capable of agglutinating Gaertner group bacilli in high dilution. These facts point to the view that old infection with Gaertner group bacilli had taken place.

The general result of the investigation is in favour of the view that while rats are liable to be infected with Gaertner group bacilli and to be ill in consequence these bacilli are not natural intestinal inhabitants. If this be accepted it may be stated that this group of bacilli are not natural intestinal inhabitants, of any known animal species.

Rats infected with Gaertner group bacilli may serve as a means of infecting meat with these bacilli and may possibly in this way be a cause of meat poisoning outbreaks.

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