RATS AND SALMONELLA GROUP BACILLI.

By WILLIAM G. SAVAGE, M.D., B.Sc. and P. BRUCE WHITE, B.Sc.

In connexion with our investigations upon the methods of causation of food poisoning outbreaks we considered it advisable to examine a further series of rats.

The rats examined were all caught in slaughter-houses and fall into two groups:

Series I. From slaughter-houses used for animal killing for human food.

Series II. From a large knacker's yard.

The rats were caught and killed and examined within 24 hours at the laboratory. In no instances were any rats found dead from disease available for examination. They were obtained from six different slaughter-houses for Series I and from one knacker's yard for Series II.

The examination was an extensive one and consisted of a careful macroscopic examination of the organs, cultural examination of the spleen, liver, heart-blood and intestines and, in most cases, a serological examination of the blood against known members of the Salmonella group.

Ninety-six rats in all were examined, 70 in Series I and 26 in Series II.

In 46 cases of Series I there were no macroscopic lesions and the internal organs appeared healthy. In 24 cases some deviations were noted, some being due to injury, while the commonest were white spots on the liver. They were not specially associated with the presence of Salmonella strains or agglutinins and were probably due to animal parasites, but the point was not investigated. In Series II 13 rats were normal.

Salmonella strains were isolated from six rats, all from Series I, and all from one particular slaughter-house.

They were isolated between December 9 and 31, 1921, and earlier examination of 14 rats from the same slaughter-house were all negative. The six strains were all alike and culturally and serologically identical with *B. enteritidis*.

Two strains somewhat resembling this group were obtained from the rats of Series II but they fermented salicin and did not produce alkalinity in milk.

The agglutination reactions of the blood of the rats are of interest. Tested by the macroscopic method in dilutions of 1 in 20, 1 in 40 and 1 in 100, 46 rats of Series I and 20 of Series II were examined. In no instance were any positive

reactions obtained with *B. aertrycke* or *B. suipestifer* strains but the following positive results were obtained with *B. enteritidis*:

	Series I	Series II
Positive up to 1 : 40 only	4	4
,, ,, 1 : 100	3	1
,, ,, 1 : 200 or 1 : 250	3	0
,, ,, 1 : 400	1	0
,, ,, 1 : 800 or higher	4	0
Entirely negative	31	15

It is of interest to consider the agglutination reactions of the rats from which *B. enteritidis* was isolated and this is shown in the following table:

Rat	Organs from which isolated	Highest agglutination with *B. enteritidis*
40	Spleen only	1 : 100
44	Liver, small intestine	Negative
58	Spleen, liver, heart-blood	1 : 400
59	Spleen, liver	1 : 800
61	Small intestine only	1 : 1000
64	,, ,,	1 : 250

It will be noticed that in only one instance was *B. enteritidis* isolated without the presence of a high agglutinative reaction of the blood to this organism. This negatives the view that they were either natural intestinal organisms or were present as passive carriers without infection. We have to do with a definite infection of the rat.

A positive reaction was obtained in 20 out of 66 rats examined, or 30 per cent. We are of opinion that these positive results are reliable evidence of old or active infection with *B. enteritidis*. They suggest that a considerable proportion of these slaughter-house rats are at one time or another infected with *B. enteritidis*.

Two of the strains were tested for virulence by the subcutaneous injection of mice and both were highly virulent.

The virulence by feeding was tested upon a six weeks' old kitten, fed with 10 c.c. of a two-days' old broth culture. No ill-effects noted for four days, then some yellow diarrhoea and the kitten very quiet and apparently ill. *B. enteritidis* could not be recovered from the excreta while next day the kitten was apparently well. Animal appeared well but died 20 days after the feeding. No naked eye lesions and internal organs and heart-blood sterile, but *B. enteritidis* readily isolated from the small intestine.

Very similar results were obtained with a purchased culture of "Liverpool Virus." This was rather more virulent to a kitten (850 grms.) by feeding, the animal being ill after four days and dead on the fifth. A typical *B. enteritidis* was isolated from the small intestine, spleen and heart-blood, but not from liver or kidney. It fermented glucose rather slowly but otherwise was true to type.

Several visits were paid to the slaughter-house and very careful inquiries made to try and elucidate the source of infection of the rats with *B. enteritidis*.

No rat virus had been used by the butcher on the premises for a year or more, nor could its usage be traced in any of the neighbouring premises. Visits

to the local chemists elicited that while none had been sold recently, the Co-operative Society in the vicinity had been supplied periodically some time previously with "Liverpool Virus."

It seemed probable that no rat virus had been used recently but it is obviously impossible to exclude this as the original source of infection.

It is of interest to compare these results with two other investigations which one of us has carried out.

In 1913 Savage and Read examined 41 rats collected at Weston-super-Mare, mostly from refuse tips. No Salmonella strains were isolated from the intestinal contents, but five strains of *B. enteritidis* were obtained from the spleen and liver of different rats. In two instances Danysz's virus (*B. enteritidis*) had been distributed quite recently, while two and a half years previously the refuse tips had been extensively dosed with this virus. They concluded that it was probable that this virus was the primary source of infection.

Savage in 1917 examined a further series of 48 rats from Bristol and Avonmouth which were collected for examination for plague infection. None showed true Salmonella organisms but five closely allied para-Gaertner strains were isolated which were culturally identical with some *B. suipestifer* strains but did not agglutinate with that organism and were non-pathogenic to a rabbit. Tested serologically only three of the rat blood samples showed positive agglutination and those only up to 1 : 50 dilution (microscopic); one from a ship, one from a food ship, one from a slaughter-house.

Observations.

It is fairly evident that Salmonella strains are not natural intestinal inhabitants of the rat but that they may be present in a good many animals. Whether these are derived from bacterial bait or from natural infection is not clear. The essential points we wish to emphasise are that in 6 out of 96 rats *B. enteritidis* was isolated, that they were highly virulent, that they differed in no particular from the strain isolated from food poisoning outbreaks and that these rats were from slaughter-houses in which they would have opportunities to infect meat.

It will be noted that they were fatal to a kitten by feeding and that in three out of the six cases they were isolated from the intestine of the rat and therefore would be shed into the faeces and quite possibly upon food in the slaughter-houses. Further, many of the rats showed positive agglutinins for *B. enteritidis* which we regard as evidence of old infection, so that at any one time there are likely to be rats actively excreting these bacilli in the slaughter-houses.

It is not easy to ascertain in any outbreak of food poisoning if infection from rats was the source of infection so it is perhaps not surprising that more outbreaks have not been traced to this source. Particulars of human outbreaks from rat virus are given and discussed in Savage's *Food Poisoning and Food Infections*, 1920, while Willfühl and Wendtlandt (1921, *Zeitschr. f.*

Hygiene und Infektionskr. XCIV. 192) recently described three outbreaks which they ascribe to "Ratin."

We see no reason to regard these strains of rat virus culturally identical with *B. enteritidis* as other than identical with the same organism as isolated from food poisoning outbreaks. The only difference seems to be a modified virulence which may readily be increased again.

We cannot regard as other than disquieting the usage of such bacterial viruses to kill rats which may and often do gain access to foods used for man.

In any case, whether our positive cases were due to old infections with rat virus or not they emphasise the need for preserving food from being contaminated with rats and the need for steps to keep rats away from slaughter-houses.

This investigation was carried out under the auspices of the Ministry of Health and Medical Research Council and is part of an extensive enquiry into the whole question of the etiology of food poisoning and food infections.