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Salmonella in the American cockroach: evaluation of vector potential through dosed feeding experiments

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SUMMARY

Restrained American cockroaches, *Periplaneta americana* L., were fed graded doses of *Salmonella typhimurium* ranging from $1{\cdot}6 \times 10^3$ to $2{\cdot}0 \times 10^6$, and their faeces assayed daily for the pathogen. Only 4 specimens out of 117 demonstrated multiplication of salmonellas, which was unrelated to size of input. When data of persistence regardless of actual numbers were expressed as percentage *Salmonella*-positive faecal-days, and these transformed to probits, a graph of percentage-positive faecal-days versus log dose allowed a calculation of the CD50, or contaminative dose required for 50% of the faecal-days to be infective. The CD50 for this cockroach species was $1{\cdot}4 \times 10^6$ *Salmonella*.

INTRODUCTION

Cockroaches have long been regarded as possible vectors of human enteropathogens owing to their indiscriminate feeding on sanitary wastes and human food. This suspicion has been strengthened by reports of natural isolations of pathogens from cockroaches found in human environments (Mackerras & Mackerras, 1949; Graffar & Mertens, 1950; Fernandez & Lembke, 1973). Experimental vector studies have generally dealt with the faecal persistence in cockroaches often fed unknown doses of pathogens, and there have been few attempts to quantitate outputs and thus establish the occurrence of multiplication in the insect gut. These prior studies lacked a standardized treatment of data whereby the input dose of pathogens ingested by cockroaches and the resulting fate in the gut could be meaningfully expressed. Our goal in this investigation was to define the vector ability of the American cockroach, *Periplaneta americana* L., in terms of its potential for propagative versus mechanical transmission of *Salmonella typhimurium* and establish an index of pathogen dissemination which could be used to compare the results obtained using *Periplaneta*, with other insect species.

MATERIALS AND METHODS

Periplaneta adults were obtained from laboratory colonies raised in an insectary on rat chow and fruit for the past 5 years. By modifying the method of Wedberg & Clark (1947), they were immobilized with CO_2, and restrained by placing a drop

Table 1. *Summary of quantitative faecal output studies*

Salmonella input	No. of specimens	Maximum *Salmonella* excretion	Maximum length of *Salmonella* excretion (days)
$1{\cdot}6 \times 10^3$	25	$3{\cdot}6 \times 10^2$ [S]	2
$1{\cdot}9 \times 10^4$	28	$1{\cdot}6 \times 10^7$ [S]	2
$2{\cdot}3 \times 10^4$	23	$6{\cdot}2 \times 10^4$ [C]	4
$4{\cdot}8 \times 10^5$	20	$5{\cdot}6 \times 10^5$ [C]	7
$2{\cdot}0 \times 10^6$	21	$5{\cdot}2 \times 10^6$ [C]	16

The third column lists cumulative outputs for a single specimen [C], or single 5-hr. outputs [S].

of molten paraffin on the end of corks, and attaching the specimens by the pronotum when the wax solidified (Plate 1). Such a procedure minimized the possibility of recontamination during the experiments.

A recently isolated culture of *Salmonella typhimurium* was obtained from the Illinois Department of Public Health Laboratories, and a strain resistant to 1 mg./ml. of streptomycin sulphate was developed in our laboratory. This allowed enumeration of *Salmonella* on MacConkey's agar plus streptomycin while suppressing the normal faecal flora of the cockroach. Eighteen-hour BHI broth cultures of *Salmonella* were appropriately diluted in a sterile solution of powdered milk and fed to mounted cockroach specimens after a one-day starvation, by means of a 10 μl. Hamilton syringe. After the initial infective meal, daily feedings to repletion were from a solution consisting of 5 % powdered milk, 1 % yeastolate, 0·5 % sucrose, and 0·5 % peptone; feeding generally stimulated defaecation.

After feeding, faecal collections were made during a 5-hr. period each day by positioning vials containing 2 ml. of sterile physiological saline under each specimen (Plate 1). Tests demonstrated that no significant increase or decrease in numbers of salmonellas occurred in the saline. Immediately after the 5-hr. sampling period, the saline was diluted and plated on MacConkey's agar, incubated at 37° C. for 24 hr., and the colonies counted and checked on triple sugar iron (TSI) agar.

RESULTS

Two strategies were used to evaluate *Salmonella* outputs. The first considered actual numbers of the pathogen in faeces to determine if multiplication had taken place within the gut. Table 1 summarizes the results of faecal output analysis in the 117 total specimens constituting 5 input groups. Figs. 1 and 2 are examples of daily numbers of *Salmonella* following the infective meal in two of those groups. Using the criterion of at least a twofold increase of salmonellae, multiplication was shown to be an infrequent phenomenon. Only two specimens demonstrated significantly higher counts in single defaecations and two others had cumulative *Salmonella* outputs higher than inputs. Higher inputs generally produced higher outputs; two exceptions in the $1{\cdot}9 \times 10^4$ group were in excess of 10^7 (Fig. 2). Also, as input numbers increased, so did the number of days in which *Salmonella* persisted in the faeces (Table 1). There were no significant differences in infectivity between males and females in each of the 5 input groups.

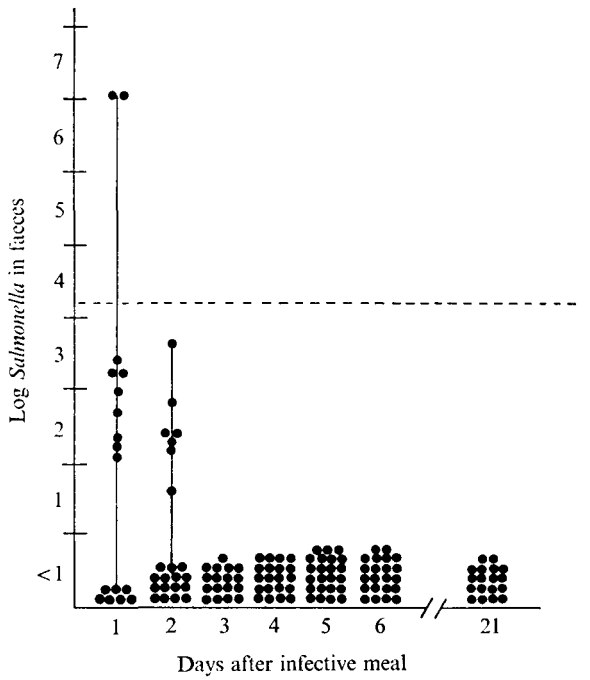

Fig. 1. Daily levels of *Salmonella typhimurium* in the faeces of *P. americana* fed $1{\cdot}9 \times 10^4$ microorganisms. Points are 5-hr. outputs of individual specimens. Dotted line shows the input level.

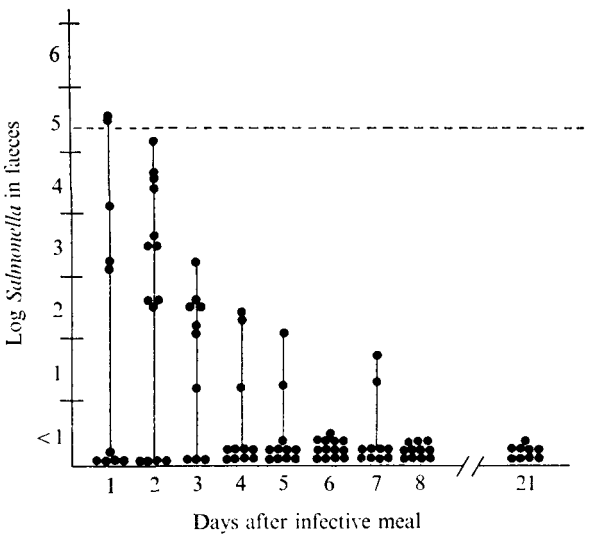

Fig. 2. Same as Fig. 1, but with an input of $4{\cdot}8 \times 10^5$ *Salmonella*.

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Table 2. *Derivation of percent* Salmonella*-positive faecal days, and transformation to probits*

Salmonella input	Percentage *Salmonella*-positive faecal-days	Probit percentage *Salmonella*-positive faecal-days
$1{\cdot}6 \times 10^3$	(1/145) 0·69	2·54
$1{\cdot}9 \times 10^4$	(17/258) 6·59	3·49
$2{\cdot}3 \times 10^4$	(15/258) 5·81	3·43
$4{\cdot}8 \times 10^5$	(29/116) 25·00	4·33
$2{\cdot}0 \times 10^6$	(80/126) 63·49	5·35

Numbers in parentheses show number of *Salmonella*-positive faecal-days/number of total faecal-days over the 21-day assay period.

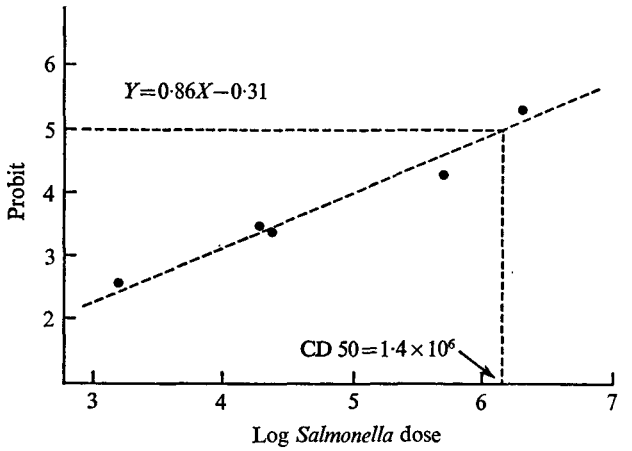

Fig. 3. Plot of probit percentage *Salmonella*-positive faecal-days versus log dose. The CD 50 of $1{\cdot}4 \times 10^6$ is the dose required to produce infected faeces in 50% of the faecal days over a 21-day period.

A second method of evaluating data assumed that any size inoculum of *Salmonella*, even a small dose, can multiply on suitable food, provided there is a sufficient period of external incubation. We defined as a 'contaminative dose' an oral input of *Salmonella* which produces any output in the faeces. By administering graded doses, a standard biological assay determines an input for which 50% of the specimens excrete *Salmonella*, but in this case the size of the input affects both the number of specimens infected as well as the length of time during which the pathogen is excreted. The concept, 'percentage-positive faecal-days', takes into account both these factors, and is based on the number of faecal-days in which *Salmonella* is recovered during the course of an experiment divided by the total number of faecal-days of data. Table 2 presents the results for each dose. Next, the percentage *Salmonella*-positive faecal-days were transformed to probits, and these plotted versus the log *Salmonella* dose (Fig. 3). With the line which resulted (chi-square: $P > 0{\cdot}60$) we can derive a value for a contaminative dose (CD 50) which results in 50% of the faecal days for a 21-day period being *Salmonella* positive. This value, $1{\cdot}4 \times 10^6$, is a convenient index for comparison with other

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insect species implicated in *Salmonella* transmission, as well as for other pathogens suspected to be spread by *Periplaneta*.

DISCUSSION

Prior investigations have demonstrated that the cockroach varies in its ability to host salmonellas acquired orally, due largely to a failure to measure input and output. Using four species of cockroaches and five species of *Salmonella*, Mackerras & Pope (1948) fed unknown doses to a small number of restrained specimens and reported infective excretions for as long as 40 days, in the case of *S. adelaide* administered to *Nauphoeta cinerea*. With *P. americana* fed approximately 10^9 *S. oranienburg*, Olson & Rueger (1950) collected infected faeces for 10 days; German and Oriental cockroaches produced positive faeces for 12 and 20 days, respectively. Jung & Shaffer (1952) fed graded doses of *S. typhimurium* and *S. montevideo* by allowing *P. americana* to ingest artificially infected human faeces. Persistence in the gut was noted for at least 7 days when 10^9 or more cells were ingested. Using two species of *Blaberus*, Krieg, Wedberg & Penner (1959) recovered salmonellas for 17 days after feeding 3×10^9 bacteria. When fed unmeasured doses of *S. typhosa*, American cockroaches passed the pathogen in faeces for 6 days (Rueger & Olson, 1969).

Unknown inputs in most of these investigations makes it impossible to generalize on the vector capacity of cockroaches, since our data show that persistence in faeces is dose-related. The question of whether multiplication of *Salmonella* occurs within the gut can only be answered if quantitative studies are undertaken. Our data suggest that the cockroach gut is not a suitable microenvironment for establishment of *S. typhimurium*. Multiplication occurred in only 4 out of 117 specimens and was unrelated to size of input.

The method of calculating the CD50 by means of a probit transformation is a variation of the dose–response experiments commonly used to determine LD50 (lethal dose) in pharmacological studies. The use of the faecal-day in the treatment of data allows the results of several experiments to be incorporated into the CD50 as a single index of vector potential. Such an index conveniently expresses the persistence of salmonellas in the faeces and can be used to compare the fate of different pathogens, as well as other insects presumed to be implicated in oral-faecal transmission of microorganisms. If *Periplaneta* normally ingests 0·02 to 0·1 g of faeces (Jung & Shaffer, 1952), perhaps the CD50 is too high for the insect to realistically encounter in nature, since when dogs were fed 2×10^{10} salmonellas, their faeces contained an average of 8×10^4 *S. typhimurium* per gram (Greenberg, 1964).

Factors contributing to the elimination of salmonellas from the cockroach most likely relate to the physical wash-out resulting from normal gut motility, as well as microbial antagonisms by the indigenous gut flora. When cockroaches were fed 0·025 ml. of a 0·1% uranine dye solution followed by daily feedings of milk solution, the gut was found to be completely cleared of demonstrable dye by the ninth day. Thus, even an inert solution fed in this concentration persists longer than the 7 days observed when $4{\cdot}8 \times 10^5$ salmonellas were ingested (Table 1).

We previously demonstrated the impact that the gut flora of adult house flies and green blow flies can have on *Salmonella* persistence (Greenberg, Kowalski & Klowden, 1970). Such a mechanism contributing to pathogen elimination by the resident flora is likely to occur in the cockroach as well. There are no specific microbial species associated with the cockroach digestive tract, and when raised aseptically, German cockroaches compared favourably in terms of longevity, developmental rates, and reproduction, with a conventional colony (Benschoter & Wrenn, 1972). Bitter & Williams (1949) found seasonal variations in the enteric flora of wild cockroaches; Burgess, McDermott & Whiting (1973) concluded that the internal environment of the insect merely reflects the microorganisms of their external environment. These observations suggest that a feeding experiment utilizing gnotobiotic specimens would best illustrate the role of the resident flora in suppressing salmonellas, and may explain the few instances of multiplication we observed in our experiments.

Another important consideration is the effect of immobilization on the normal physiological processes of the insect. Cockroaches can become paralysed from stress due to restraint (Beament, 1958) and mechanical stimulation (Cook & Holt, 1974). The mean longevity of our specimens during the 21-day assay period was 15·2 days; immobilization undoubtedly had an effect on their lifespans, but the effects of such stress on vector potential are unknown.

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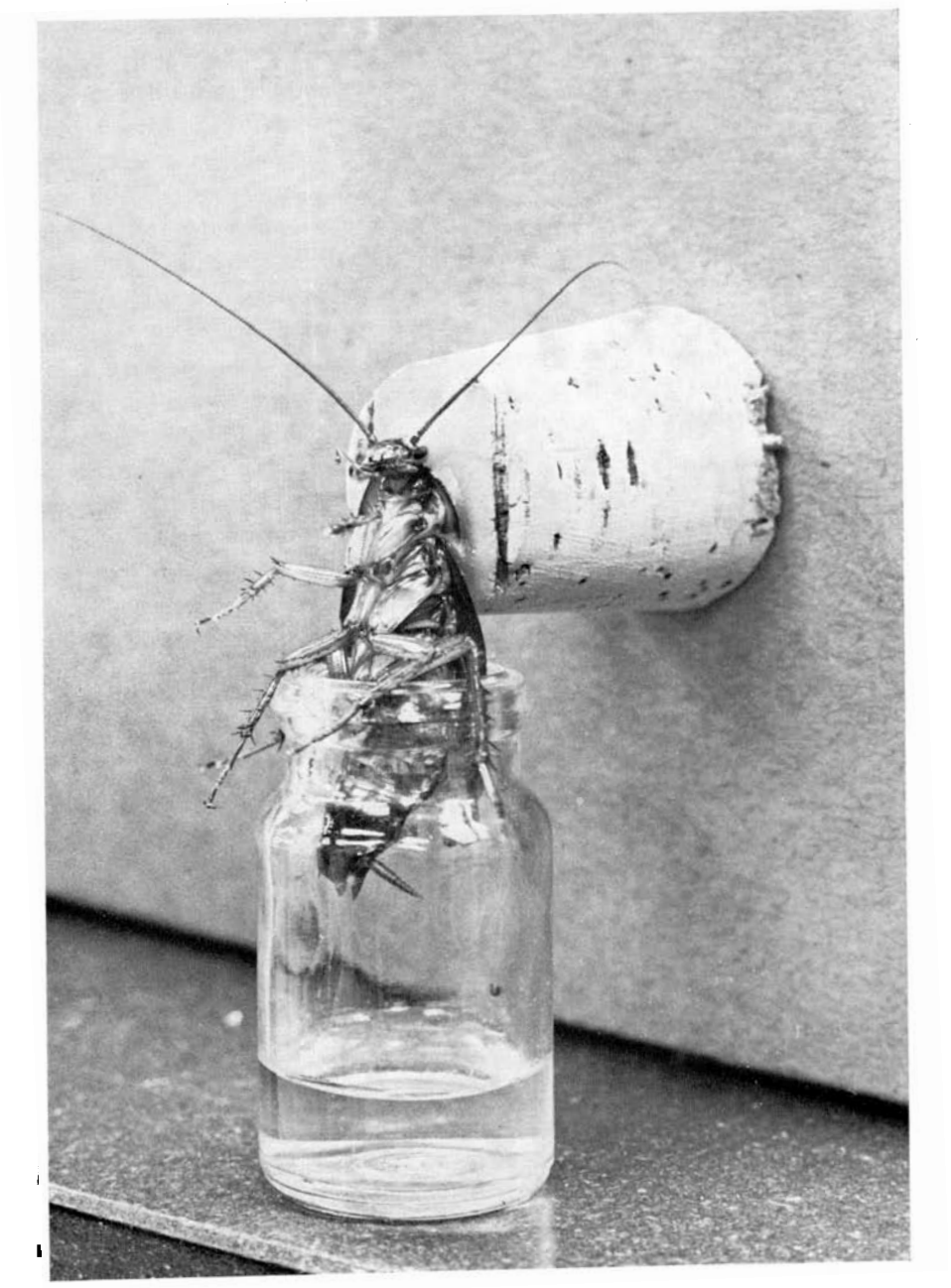

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EXPLANATION OF PLATE

Method of restraining cockroaches and collecting faeces. Hind legs straddled the vial to minimize recontamination.