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Effects of antibiotics on the survival of *Salmonella* in the American cockroach

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SUMMARY

The faecal survival of *Salmonella typhimurium* orally administered to restrained American cockroaches maintained on an antibiotic diet was investigated. Significant reductions in median numbers of total aerobic faecal microorganisms were noted in insects fed antibiotics daily, and when *Salmonella* was introduced to this modified micro-environment, it persisted for 44 days in all specimens. Multiplication of the pathogen was also observed in these insects, with numbers often exceeding 10^3 times that of the initial input. This differs significantly from our previous results showing that the pathogen is unable to multiply in conventional cockroaches. Attempts to restore the normal flora by feeding a faecal suspension from untreated cockroaches resulted in a decrease in numbers of *Salmonella* excreted, but did not result in their elimination. Carcasses of infected cockroaches retained viable *Salmonella* for at least 60 days *post mortem*, or 104 days after the infective meal.

INTRODUCTION

In the vast majority of insects, extracellular microorganisms found within the digestive tract merely reflect the bacterial species populating the external environment. Except for a few insect species, notably termites, the gut flora is purely adventitious and is not necessary for the survival of the host. This is particularly true of synanthropic cockroaches; surveys of the microbial inhabitants of these insects have failed to demonstrate distinctive bacterial components, and when reared aseptically, their longevity, reproductive rates, and nutritional requirements are not affected (Roth & Willis, 1960; Benschoter & Wrenn, 1972).

The ability of the resident gut flora to suppress the establishment of invading pathogenic microorganisms has been demonstrated in several hosts, including mice (Bohnhoff, Drake & Miller, 1954; Freter, 1956; Meynell & Subbaiah, 1963), guinea pigs (Freter, 1956; Formal *et al.* 1961), and house fly larvae and adults (Greenberg, 1959; 1966; Greenberg, Kowalski & Klowden, 1970). Even microorganisms usually considered to be innocuous for conventional animals have been shown to be damaging in the absence of a normal intestinal flora (Luckey, 1963). In view of our previous data showing that *Salmonella typhimurium* does not survive

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Table 1. *Composition of the antibiotic-milk solution used to feed immobilized cockroaches*

Component	Grams/100 ml
Powdered milk	5·0
Yeast extract	1·0
Bacto-peptone	0·5
Sucrose	0·5
Penicillin G	10000 units
Streptomycin sulphate	0·1

well in the gut micro-environment of laboratory cockroaches (Klowden & Greenberg, 1976), the present investigation was undertaken to determine the effects of altering the composition of the cockroach gut flora with antibiotics on the subsequent faecal persistence of orally administered *Salmonella*.

MATERIALS AND METHODS

Adult *Periplaneta americana* L. were derived from long-term laboratory colonies, and were reared and immobilized on corks to minimize recontamination as described previously (Klowden & Greenberg, 1976). The same strain of *Salmonella typhimurium*, resistant to 1 mg/ml of streptomycin sulphate, was also used, allowing us to suppress the growth of the normal faecal flora of the cockroach on MacConkey's agar plus streptomycin for the enumeration of the pathogen alone. Non-selective brain heart infusion agar (BHI) was used for determining numbers of total aerobic flora.

In the first experiment, a group of 28 restrained cockroaches was tested to determine the effectiveness of the antibiotics in altering the insects' gut flora. Half of this group was fed the antibiotic-milk diet (Table 1) daily for 21 days, and the other half was maintained on the same diet without antibiotics. Faecal collections were made during a 5-h period immediately after feeding by positioning vials containing 2 ml of sterile physiological saline beneath each insect; the faecal suspension was plated on BHI agar, and counts of total aerobic microorganisms present were made after 24 h incubation.

The second experiment consisted of 24 *Periplaneta* maintained on the antibiotic-milk diet and fed 6×10^4 *Salmonella typhimurium*. Faecal assays were plated on MacConkey's agar plus streptomycin to indicate the numbers of *Salmonella* in each defecation and, after incubation, the colonies were tested in triple sugar iron agar to verify their identity. On day 10, the group was subdivided, with half the specimens (Group A) continued on the antibiotic diet, and the other half (Group B) fed a suspension of fresh faeces from untreated cockroaches diluted in sterile milk solution to attempt to restore the normal flora, followed by daily feedings of milk solution without antibiotics. Assays of *Salmonella* continued over a period of 44 days for Group A; Group B samples were curtailed after 30 days due to an unusually high mortality.

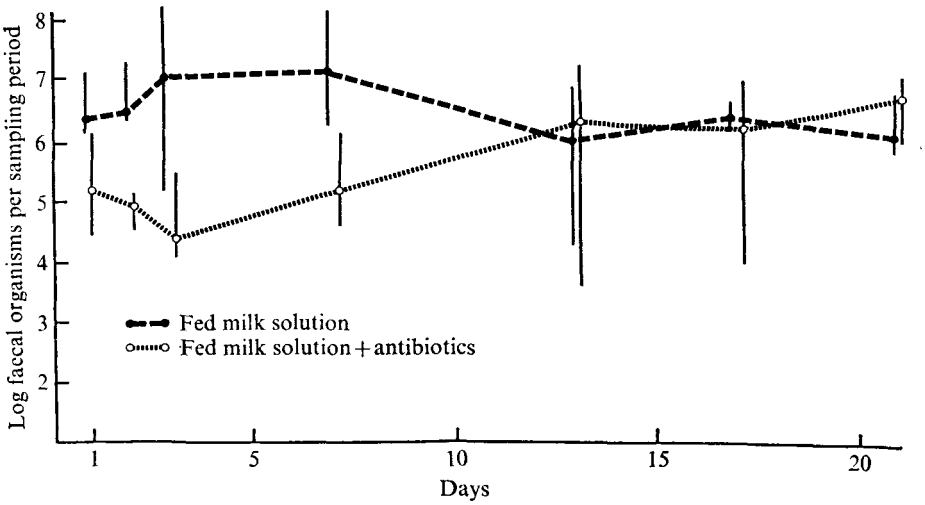

Fig. 1. Numbers of total aerobic faecal micro-organisms in cockroach excretions during a 5-h sampling period. Cockroaches were maintained on the standard milk diet with or without antibiotics. Points indicate median values; vertical lines represent the ranges.

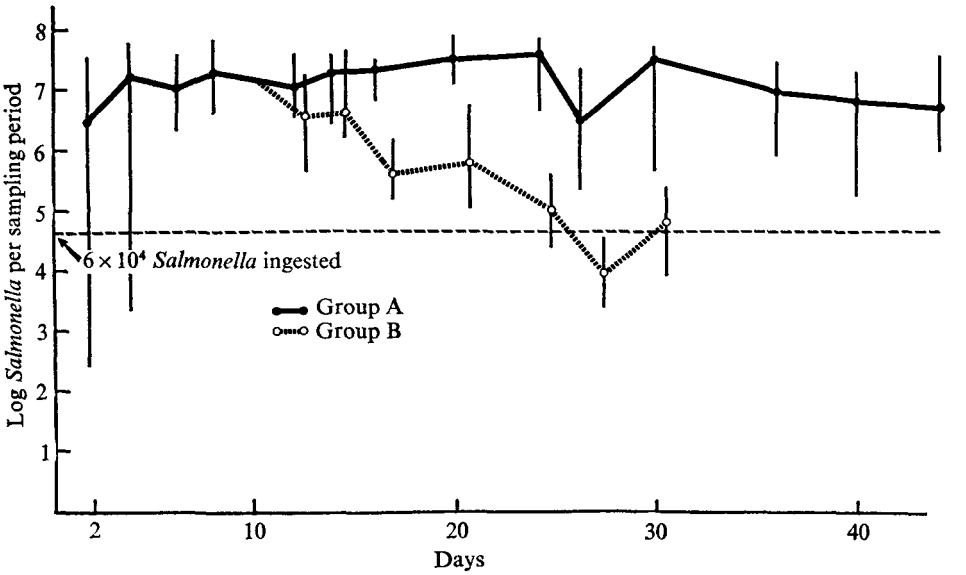

Fig. 2. Numbers of *S. typhimurium* in cockroach excretions during a 5-h sampling period. All insects were fed 6×10^4 *Salmonella* on day 0 (horizontal dashed line); Group A cockroaches were maintained on the standard milk diet including antibiotics for 44 days. Group B cockroaches were taken off antibiotics on day 10 and fed a suspension of fresh cockroach faeces. Points indicate median values; vertical lines represent the ranges.

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Table 2. *Post-mortem survival of salmonellas in cockroach carcasses*

Days post-mortem	*Salmonella*/specimen
18	$2{\cdot}7 \times 10^7$
38	$1{\cdot}2 \times 10^6$
54	$4{\cdot}3 \times 10^6$
60	$7{\cdot}2 \times 10^5$

RESULTS

Figure 1 shows that the median numbers of aerobic faecal organisms remained fairly constant over the 21-day period when cockroaches were maintained on the milk solution without antibiotics, remaining at approximately 10^6 bacteria per 5-h sampling period. Also shown in Fig. 1 are counts in those specimens fed the antibiotic-milk diet; these experienced a gradual decline in numbers of faecal organisms during the first three days, followed by a gradual recovery by day 13. Thereafter, both groups showed equivalent numbers of total aerobic micro-organisms in excretions.

When cockroaches were maintained on the antibiotic-milk diet and fed 6×10^4 *Salmonella*, the pathogen multiplied and was excreted in amounts up to 10^3 times that of the initial input for 44 days (Fig. 2, Group A). Group B, taken off the antibiotic diet on day 10 and fed a faecal suspension, continued to excrete smaller numbers of *Salmonella* for 20 more days (Fig. 2, Group B). In both groups, all cockroaches excreted *Salmonella* until they died. A post-mortem assay for *Salmonella*, performed by triturating whole insects in sterile saline, showed that the pathogen was still viable in cockroach carcasses for at least as long as 60 days after death, which was 104 days after the initial feeding (Table 2).

DISCUSSION

The best strategy to study the effects of a microbial flora on the establishment of a pathogen would be to rear the host in the absence of such a flora. These techniques have been well established for insects (Greenberg, 1970), and we have studied the fate of enteroviruses in *Periplaneta* embryonic tissue aseptically obtained and cultured (Klowden & Greenberg, 1974). However, the rearing of gnotobiotic adult *Periplaneta* was deemed impracticable because of the relatively long period of time (approximately 1 year) to reach maturity. Altering the composition of the gut flora with antibiotics was therefore chosen as an alternative. Banerjee & Raychaudhuri (1967) reported the complete elimination of bacteria from the digestive tract of *Periplaneta americana* maintained on chloramphenicol, but there were some indications of toxicity of the drug. Banerjee, Bhowmik & Raychaudhuri (1971) reported that a combination of chloramphenicol, terramycin, and streptomycin successfully reduced bacterial populations, but neither the amount ingested nor the numbers of remaining bacteria were specified. We observed an initial decline in the numbers of bacteria attributable to antibiotics, but the total aerobic flora regained its former levels after 13 days despite continued

feeding of antibiotics (Fig. 1). Although a census of the bacterial species was not attempted, we suspect that populations in the gut are profoundly different in the groups maintained on the diet with and without antibiotics, although the total numbers are similar after day 13.

Our results demonstrating multiplication and long-term persistence of *Salmonella* in *Periplaneta* which were maintained on antibiotics are consistent with the contributions of the normal flora to resistance against invading pathogens observed in other animals. Bohnhoff *et al.* (1954) demonstrated that mice treated with streptomycin had an ID50 for *Salmonella enteritidis* 10^4 times lower than that of conventional mice. They attributed the increase in susceptibility to a disturbance of the normal intestinal microflora caused by the antibiotic. Freter (1956) established *Shigella flexneri* in mice given erythromycin and streptomycin. Controls which were not fed antibiotics failed to demonstrate *Shigella* in faeces, and the introduction of *E. coli* to treated mice resulted in the gradual elimination of *Shigella* from the gut. In fly larvae, there are many examples of human bacterial pathogens failing to survive through metamorphosis when a competing flora was also present, but in monobiotic flies *Salmonella typhimurium* was not eliminated (Greenberg, 1966). *Proteus mirabilis* was shown to be a specific antagonist of *Salmonella* in both larval and adult flies (Greenberg & Miggiano, 1963; Greenberg *et al.* 1970).

The laboratory cockroaches used in this study showed a dramatic change in their susceptibility to *Salmonella* when given antibiotics. The pathogen's persistence for 44 days in all insects fed 6×10^4 *Salmonella* greatly exceeds the persistence we observed in conventional cockroaches fed $2{\cdot}3 \times 10^4$ and $4{\cdot}8 \times 10^5$ bacteria, in which positive outputs were recovered for only 4 and 7 days respectively (Klowden & Greenberg, 1976). The cessation of antibiotic feeding and the re-introduction of faecal bacteria led to a decline in the numbers of *Salmonella* excreted (Fig. 2, Group B), but this apparently was insufficient to fully restore the populations of bacteria which suppress the pathogen. The trend towards elimination of *Salmonella* might have continued had the specimens survived longer.

Although cockroaches are unlikely to encounter antibiotics which may modify the composition of their gut flora in nature, the natural seasonal and environmental variations in bacterial components may make these insects more susceptible at certain times and places than those experiencing the relatively uniform rearing conditions of an insectary. Seasonal variations in enteric inhabitants of the cockroach were reported by Bitter & Williams (1949), who noted that *Proteus* sp. disappeared from specimens captured in the winter. Brooks (1963) pointed out that Wedberg, Brandt & Helmboldt (1949) and Briscoe, Moore & Puckett (1961) both examined the microbial species of *Blaberus craniifer*, but only 2 of the 17 bacteria reported from both groups of investigators were the same. This variable nature of the bacteria found in cockroaches is further emphasized by Burgess, McDermott & Whiting (1973), and may partially explain the often negative reports of experimental persistence of pathogens in these insects. The microbial populations of the cockroaches used in this study were not identified, and the specific antagonists are not known. However, Miller & Bohnhoff (1963), examining

the components of the intestinal flora of mice treated with streptomycin, found that gram-negative bacilli were significantly reduced in numbers, and the disappearance of the anaerobic *Bacterioides* from the enteric flora had the most influence on susceptibility to *Salmonella*.

The post-mortem persistence of *Salmonella* (Table 2) in the carcasses of cockroaches infected before their death is important in view of the minimal levels of 'filth' composed of insect parts and rodent hairs, which food processors are allowed, by federal law, to include in their foods. Cockroach remains are undoubtedly major contributions to this tolerable filth. Unpasteurized foods, such as spices and packaged cereals, might be 'seasoned' with a portion of infected cockroach carcass, and become a route of *Salmonella* dissemination. The incorporation of contaminated cockroach debris into the environment of the home, restaurant, and food processing plant is another avenue which has not been explored.

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