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Laboratory transmission of Enterobacteriaceae by the oriental cockroach, *Blatta orientalis*

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SUMMARY

Methods of maintaining and feeding and of infecting cockroaches with pathogenic organisms were investigated.

Cockroaches fed on known concentrations of *Escherichia coli* O119, *Esch. coli* O1, Alkalescens Dispar O group 2 and *Shigella dysenteriae* 1 (Shiga's Bacillus) were maintained in Petri dishes. The effect of various diets on the survival of these organisms within the hind-gut and faeces was observed. With a 'normal' diet of gruel *Esch. coli* O119 was isolated for up to 20 days, *Esch. coli* O1 for 17 days and ADO 2 for 15 days. *Sh. dysenteriae* 1 was isolated only sporadically to the third day.

INTRODUCTION

The domesticated cockroaches, *Blatta orientalis*, *Periplaneta americana* and *Blattella germanica* have been shown to feed readily on faeces, sputum, skin scrapings and other human waste, and a wide variety of human food-stuffs (Roth & Willis, 1967). Burgess, McDermott & Whiting (1973) investigated the normal aerobic bacterial flora of the hind-gut of *Blatta orientalis* and refer to a number of instances where pathogenic bacteria have been isolated from cockroaches, both under natural conditions and in laboratory passage experiments.

METHODS AND MATERIALS

Maintenance of cockroach colonies

Later stage nymphs and adult cockroaches were housed individually in sterile disposable Petri dishes. Three series of cockroaches, 59 cockroaches in each series, were fed as follows.

Series A were given only water each day; these are termed 'starved'.

Series B were provided each with half of a 9 cm. filter paper for shelter, and were fed on a 'normal' diet of a sloppy chicken food gruel emulsified in water.

Series C were kept on sterile MacConkey plates agar downwards.

Plates were changed and individuals fed or watered every 24 hr.

Infection of cockroaches with organisms

A suspension containing a known number of organisms determined by the technique of Miles & Misra (1938) was used. After 72 hr. starvation, each cockroach was placed overnight in an incubator at 35° C. Next morning, one drop of a known

concentration from a 50-drop Pasteur pipette was placed with the cockroach in a sterile Petri dish. The majority of cockroaches drank readily. Only those which took the full quantity were used subsequently. Each series of examinations included ten uninfected cockroaches as controls.

After ingestion one cockroach from each of the three feeding groups was killed daily and the hind-gut examined for the presence of the specific organisms. The cockroach was dissected under sterile conditions, the hind-gut being removed and emulsified in $\frac{1}{4}$ strength Ringer's solution. The emulsion was plated out on MacConkey's medium, incubated at 37° C. for 24 hr., and any growth observed.

In addition, the MacConkey plate on which the cockroach had lived and defaecated for the 24 hr. before dissection was incubated at 37° C. overnight, and any growth recorded.

During preliminary work it was suspected that the organism, or an associated toxin, was killing the infected cockroach. To check this, groups of ten cockroaches were fed as follows by the method described above.

(*a*) An overnight growth of *Esch. coli* O119 in nutrient broth (i.e. organisms with metabolic products and broth).

(*b*) Broth from an overnight growth, centrifuged, Seitz filtered, and checked for sterility (i.e. broth with metabolic products but without organisms).

(*c*) Organisms from the overnight growth washed four times and resuspended in fresh nutrient broth (i.e. organisms with broth but with no metabolic products).

(*d*) Fresh sterile broth.

A group of ten cockroaches served as a control. Only two died, one in group *c* and one in the control group. Since a damaging immobilization and forced feeding technique had been used in preliminary work, it was concluded that the method of infection, and not the organism or an associated toxin, had killed the cockroaches.

Number of organisms in infecting dose

To determine the optimum concentration of organisms, cockroaches were fed 0·2 ml. of *Esch. coli* O119 organisms at concentrations ranging from $5{\cdot}5 \times 10^3$ to $5{\cdot}5 \times 10^8$ per ml. The organism was recovered from cockroaches fed on all concentrations 3 hr. after ingestion, but thereafter, for up to 10 days, only from cockroaches fed on the highest concentration. It was decided to infect the cockroaches with a high concentration of each organism. The concentrations used were as follows: *Esch. coli* O119 2×10^8/ml., *Esch. coli* O1 $2{\cdot}8 \times 10^8$/ml. and ADO 2 $3{\cdot}1 \times 10^8$/ml. Each cockroach imbibed 0·02 ml. of the suspension (1 drop from a 50-dropper pipette).

MacConkey agar was used for isolation of *Esch. coli* and Alkalescens Dispar, deoxycholate citrate agar for the isolation of *Sh. dysenteriae*. Representative samples of colonies were selected, and shown to be pure by subculturing three times on blood plates. Identification was confirmed by Preston and Morrell's modification of Gram's stain (Preston & Morrell, 1962), followed by 'short sets' of biochemical tests consisting of Simmons' citrate agar (Simmons, 1926), TSI slopes (Report, 1958), peptone water (indole test) (Kovacs, 1928), nutrient agar and

Table 1. *Isolation of three organisms from the hind gut of cockroaches after experimental infection*

	Escherichia coli 0119	*Escherichia coli* 01	Alkalescens-Dispar 0 group 2
Series A			
Starved	13* (15)	8 (16)	8 (15)
Series B			
Gruel-fed	16 (20)	12 (17)	15 (15)
Series C			
MacConkey agar	13 (20)	10 (17)	13 (18)

* The figures show the number of days for which the organism was grown at each successive daily examination. The figures in parentheses show the last day after infection on which a positive result was obtained.

nutrient broth (for serological testing), gluconate broth (Shaw & Clarke, 1955) and urea slopes (Oxoid CM 71) (Christensen, 1946). After biochemical identification specific antisera (from David Bruce Laboratory, Everleigh) were used to confirm identification. Results will be discussed individually.

RESULTS

Esch. coli O119

The organism was isolated from the hind-gut of the cockroaches 24 hr. after infection and continued to be isolated for 15 days in the starved group (Series A) and for 20 days in the gruel fed group (Series B) and the MacConkey fed group (Series C).

Esch. coli O1

Cockroaches easily became infected with this organism and it was isolated from all three feeding series during the first day after infection. In Series A given only water, the organism disappeared on the 8th day except for one colony which was isolated on the 16th day. In Series B fed on gruel, isolations were made regularly to the 12th day, remaining fairly constant to the 17th day. In the MacConkey Series C isolations were regular to the 10th day and somewhat sporadic thereafter to the 17th day.

ADO 2

This organism also was isolated during the first 24 hr. after infection in all series. In the starved Series A it continued to be isolated up to the 8th day; beyond this a very high death rate, some 50 % higher than in either of the other starved series, renders results unreliable. In the gruel fed Series B, the organism continued to appear up to the 15th day, and in the MacConkey Series C isolations were regular to the 13th day.

These results are shown in Table 1.

Sh. dysenteriae

Groups of ten cockroaches were fed with five different concentrations of *Sh. dysenteriae* in suspension, ranging from 8×10^3 to 8×10^7 organisms/ml. Two cockroaches from each group were dissected on days 1, 3, 12, 22 and 30 after infection. The ten insects dissected and plated on day 1 were all negative; two isolations were made on day 3, one from a concentration of 80 million organisms per ml. and the other at 8000 per ml. After the third day, no isolations were made. Further work is clearly necessary on the ingestion of this organism.

DISCUSSION

Mechanism of transmission of organisms

The infecting organisms were isolated also from cockroach faecal pellets and smears deposited on the MacConkey plates some 24 hr. *before* dissection with an incidence similar to that occurring in later hind-gut cultures. Clearly it is more practical to isolate organisms from faeces provided by the cockroach than to dissect out the hind-gut.

Artificially maintained cockroaches show a number of features which would be encountered in free living cockroaches.

Cultured cockroaches, given only water, voided almost fluid faeces but retained considerable solid matter in the hind-gut which was apparent on dissection. Those fed on gruel produced numbers of faecal pellets rather than smears. When the faecal pellet was spread a confluent growth occurred. Contamination of the MacConkey plate was directly proportional to the length of time the surface had been exposed to the insect. Little contamination occurred when the cockroach was kept on the plate for only 6 hr. Ten minutes exposure produced growth rarely, whereas 24 hr. produced a good growth. Clearly, the deposition of fluid faeces on a moist medium affords the best conditions for the survival and growth of organisms. It is significant that on transferring the cockroach from one plate to another, little or no growth of organisms occurred unless faeces had been present, in which case the organisms grew in abundance. The normal flora of the cockroach was never suppressed by growth of the inoculated organism. Cockroaches fed on MacConkey medium did not produce faecal pellets and the hind-gut on dissection contained little faecal matter. The internal tissues of cockroaches in this group were stained with neutral red. Growth of organisms on the plate after incubation occurred mainly from the faecal streaks. The heaviest mechanical spreading occurred on the circumference of the plate where the insect spent most of its time, and thus dispersed its excreta.

Death rates over a period of 20 days after the initial starvation depended significantly upon diet, since no cockroach died which was fed on a 'normal' diet of gruel. There was a high death rate in those given water only (39%) and a moderate death rate in those fed on MacConkey medium (27%). Although the initial treatment of starvation and incubation resulted in a number of deaths (23%) it did not appear to affect subsequent survival.

A problem which occurred in the group fed on MacConkey medium was caused

by the organism *Serratia marcescens* which, when present in the faeces of the cockroach, grew on the plate at room temperature. The growth was re-ingested by the cockroach, defaecated onto a new plate, re-ingested and so on until eventually the concentration of *S. marcescens* appeared to kill the cockroach. This organism is present in the normal gut flora of some 6% of the insects used (Burgess, McDermott & Whiting, 1973), and under certain circumstances might present a means of biological control. In low concentrations the organism will probably fail to survive in the gut, but at higher concentrations the defence mechanism of the cockroach may be overwhelmed. This would also appear to happen in infection of the cockroach with the strains of *Esch. coli* used. Though low concentrations were quickly evacuated during the first few hours, high concentrations appeared to become established. In our investigation into the normal flora of *Blatta orientalis* we isolated *Esch. coli*, from which it must be concluded that the organism must have existed in a high concentration in the material on which the cockroach had fed.

It is worth comparing the habits of the three domestic cockroach species when considering them as possible disseminators of pathogenic organisms. *Blatta orientalis* typically shows a dislike of climbing to table tops and other raised surfaces, but will often be found on the upper floors of high buildings.

Its density is usually far lower than that of its more mobile competitor *Blattella germanica* which seems to thrive in new buildings, as well as the older buildings, boiler rooms and cellars preferred by *Blatta orientalis*. We have observed an interesting take-over of a canteen by *Blattella germanica*, which has almost completely ousted *B. orientalis* in a period of just over two years.

Periplaneta americana appears to be of little importance so far in this country. It has, however, been found by us in London sewers, contrary to statements in, for example, Cornwell (1968, p. 304).

Taking all facts into consideration it would seem that, of all cockroaches, *Blattella germanica* is likely to prove the most important potential disease vector, but the capabilities of the less mobile and less numerous *Blatta orientalis* should not be underestimated.

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