BACTERIAL ISOLATION FROM AND TRANSMISSION BY BOOPHILUS DECOLORATUS AND BOOPHILUS GEIGYI

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Abstract. Bacteria were isolated from the haemolymph of Boophilus decoloratus and Boophilus geigyi which had engorged on trade cattle in Nigeria. All the genera of the bacteria were also isolated from the eggs which they laid although some of them were missing from the larvae which eventually hatched. When clean rabbits were inoculated with some genera of bacteria and clean, laboratory bred larvae of B. decoloratus and B. geigyi fed on them, the same genera of bacteria were subsequently isolated from the haemolymph of the engorged adults, the eggs they produced and the larvae which hatched from them. Staphylococcus pyogenes and Pseudomonas aeruginosa were consistently encountered in the haemolymph, eggs and larvae of both Boophilus species while Proteus mirabilis was additionally consistent in B. geigyi.

The implication of transovarian biological transmission of pathogenic bacteria to livestock by ticks especially in countries where tick infestation is endemic, is discussed.

Although the role of ticks as vectors of some protozoan blood parasites and viruses had been well documented all over the world (Mahoney and Mirre 1974, Daglish and Stewart 1978, Akinboade and Dipeolu 1983, 1984), there is little information on their ability to transmit bacteria. Rahman and Rahman (1980) isolated bacteria of genera Staphylococcus, Escherichia, Pseudomonas, Proteus and Enterobacter from ticks of genera Boophilus, Rhipicephalus and Haemaphysalis and reported that forest workers in the Madhupur forest of Bangladesh showed a slight inflammation of areas of bite, mostly ears and armpits by the ticks. In Nigeria, Adegbeke et al. (1981) reported septicaemia with Staphylococcus pyogenes in rabbits fed with larvae of Boophilus and Hyalomma species. In this study, we report not only the isolation of bacteria from ticks but their transovarian transmission.

MATERIALS AND METHODS

With the aid of sterile forceps, engorged ticks were collected into sterile universal bottles from cattle stationed at the Veterinary Control post in Ibadan shortly before they were slaughtered. In the laboratory, those belonging to genus Boophilus were separated into sterile petri-dishes for species identification. The experiment proceeded in the following phases:

Phase I: Each tick of B. decoloratus or B. geigyi had its fourth leg amputated and a puncture made with a fine aseptic dissecting pin for haemolymph at the joint of amputation. With the aid of a platinum loop, a small innoculum of the haemolymph was streaked on Blood Agar (BA), Cysteine-lactose defiicient (CLED) and Selenite broth (SB). The agar media were left overnight at 37 °C. Identification of bacteria isolates was based on their morphological, cultural and biochemical characteristics (Cowan and Steel, 1966). The engorged ticks from which haemolymph was extracted, were incubated in the insectary maintained at 24 °C and 85 % relative humidity. Twenty (20) engorged ticks of B. decoloratus and 20 of B. geigyi collected on trade cattle on various occasions were used for this experiment.

Phase II: At the end of the oviposition period of the engorged ticks punctured for haemolymph in phase I, about 1000 eggs out of the egg-mass laid by each tick were put in a sterile mortar and crushed with sterile pestle after the addition of 2 drops of sterilized normal saline. With the aid of a platinum loop, a small innoculum was streaked on BA, CLED and SB. Identification of bacteria isolates followed as in Phase I and the remaining egg-masses from each tick were returned to the insectary for further incubation.
Table 1. Bacteria isolated from the haemolymph of adult *Boophilus* collected on trade cattle, from the eggs they laid and the larvae which hatched from them

<table>
<thead>
<tr>
<th>Species of ticks</th>
<th>Haemolymph</th>
<th>Eggs</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. decoloratus</td>
<td>No. of engorged ticks punctured</td>
<td>No. of ticks positive for bacteria</td>
<td>Species of bacteria isolated</td>
</tr>
<tr>
<td>B. geigyi</td>
<td>No. of engorged ticks punctured</td>
<td>No. of ticks positive for bacteria</td>
<td>Species of bacteria isolated</td>
</tr>
</tbody>
</table>

* Figures in bracket indicate the numbers of ticks/batches of eggs and larvae from which bacteria were isolated.
Table 2. Bacteria isolated from haemolymph of adults of *Boophilus* species which had engorged on rabbits inoculated with bacteria and from the eggs they produced and larvae which hatched from them

<table>
<thead>
<tr>
<th>Species of ticks</th>
<th>Haemolymph</th>
<th>Eggs</th>
<th>Larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of enzaged ticks punctured</td>
<td>Species of bacteria isolated</td>
<td>No. of egg batches cultured</td>
</tr>
</tbody>
</table>
| B. decoloratus  | 60         | *Staph. pyogenes* (53)  
|                 |            | *Staph. aureus* (27)    
|                 |            | *Staph. albus* (32)     
|                 |            | *Pseud. aeruginosa* (51) 
|                 |            | *Esch. coli* (18)       | 60 | *Staph. pyogenes* (48)  
|                 |            |                | |                |                | *Staph. aureus* (12)    |
| CONTROL (Rabbits without bacteria inoculation) | 40 | 0 | — | 51 | *Staph. albus* (21)  
|                 |            | *Pseud. aeruginosa* (44) 
|                 |            | *Esch. coli* (9)         | 40 | 0 | — | 60 | *Staph. pyogenes* (42)  
|                 |            |                | |                |                | *Staph. albus* (17)    | 46 | — | — | 40 | *Pseud. aeruginosa* (40) |
| B. qeigyi       | 60         | *Staph. pyogenes* (58)  
|                 |            | *Staph. aureus* (54)    
|                 |            | *Staph. albus* (39)     
|                 |            | *Bac. subtilis* (14)    
|                 |            | *Pseud. aeruginosa* (41) |
|                 |            | *Pseud. aeruginosa* (47) 
|                 |            | *Esch. coli* (38)       | 60 | *Staph. pyogenes* (54)  
|                 |            |                | |                |                | *Staph. aureus* (19)    |
|                 |            | *Staph. albus* (23)     
|                 |            | *Pseud. aeruginosa* (36) |
|                 |            | *Prot. mirabilis* (52)  | 60 | 53 | *Staph. pyogenes* (50)  
| CONTROL (Rabbits without bacteria inoculation) | 40 | 0 | — | 54 | *Prot. mirabilis* (34) |
|                 |            |                | |                |                | *Pseud. aeruginosa* (30) | 40 | 0 | — |
Phase III: About 1000 larvae which hatched from the egg mass of each tick, part of which was crushed in Phase II, were crushed in sterile saline after they were 4—5 days old and were cultured in the different media as in Phases I and II.

Phase IV: Six rabbits whose blood were negative for bacterial infection were infested with clean laboratory bred larvae (1000 on each rabbit) by the use of ear-bag. These larvae were certified clean of bacteria after the culture of part of the eggs from which they hatched and the haemolymph of adults which laid the eggs showed no bacterial growth on agar. On day 18 after tick attachment, each of the rabbit was injected peritoneally with 1 ml of a phosphate buffered bacterial solution. This solution was obtained by pooling all the larvae which carried bacterial infection as indicated by results of haemolymph puncture of adults which produced their eggs (Phase I) and egg and larval cultures (Phases II and III). The bacterial solution was then standardised by the method of Miles and Misra (1938) to contain 12 × 10^8 bacteria per ml of solution. Innoculation of bacterial solution into rabbits was made on the 18th day of tick infestation because preliminary observations in our laboratory showed that nymphs of B. decoloratus and B. geigyi normally start to engorge on rabbits around this period. Engorged adult Boophilus ticks were carefully detached with sterile forceps from the rabbits from days 21—23 after infestation. Isolation of bacteria from the haemolymph of the adults and the eggs they laid and the larvae which hatched from them were carried out as described for Phases I, II and III. Three inoculated rabbits were used for B. decoloratus and another three inoculated ones were used for B. geigyi. Two rabbits without any bacterial inoculation but infested with ticks were used as control for each of the Boophilus species.

RESULTS

Table 1 shows the results of phases I, II and III of the experiment. Three genera of bacteria i.e. Staphylococcus, Pseudomonas and Escherichia were isolated from the haemolymph of B. decoloratus adults while these, together with two genera i.e. Bacillus and Proteus were isolated from the haemolymph of B. geigyi adults. The same genera of bacteria were also isolated from the eggs laid by B. decoloratus; in the case of B. geigyi however, the genus Bacillus was not isolated from the eggs even though it was found in the haemolymph of the adult. The larval progenies of B. decoloratus yielded two of the three genera of bacteria originally isolated from the haemolymph of the adults with Escherichia missing while those of B. geigyi yielded three of the bacterial genera originally isolated from the haemolymph of the adults with Bacillus and Escherichia missing.

Table 2 shows the results of phase IV of the experiment and the bacteria isolated from various stages of ticks which engorged on rabbits that were injected with bacterial solutions are listed. The same genera of bacteria inoculated into clean rabbits were isolated from the haemolymph of the engorged adults of B. decoloratus and B. geigyi whose larvae were laboratory bred and free of bacteria. Furthermore, the two genera of bacteria found consistently in all stages of B. decoloratus and the three genera found consistently in all stages of B. geigyi as reflected in Table 1 were also found to be consistent in all stages of the ticks which engorged on the inoculated rabbits. No bacteria were isolated from all stages of ticks that engorged on control rabbits.

Generally, it was observed that Staphylococcus pyogenes and Pseudomonas aeruginosa were consistently encountered in the haemolymph, eggs and larvae of both Boophilus species while Proteus mirabilis was additionally consistent in B. geigyi. It was also observed that the cumulative number of ticks from which particular genera of bacteria were isolated decreased progressively from the haemolymph of adult ticks to their larval progenies.

DISCUSSION

Of all the bacterial isolates, only Staphylococcus pyogenes, Pseudomonas aeruginosa in B. decoloratus and these together with Proteus mirabilis in B. geigyi can be said to be transmitted transovariably since they were the only bacteria consistently isolated.
from the haemolymph, egg and larval extracts. The fact that no bacteria were isolated from the haemolymph, eggs and larval extracts of ticks which engorged on control rabbits but only from ticks which fed on trade cattle and rabbits experimentally infected with bacteria indicates that the bacteria are not part of the normal flora of the haemolymph of adult Boophilus ticks and their eggs and larvae. Hence, the source must have been the hosts which in our investigation were the trade cattle and experimentally inoculated rabbits.

The observations made from this investigation added to our knowledge of the transmission of pathogenic bacteria to domestic livestock as this is one of the few first reports of transovarial transmission of bacteria by ticks. The implication shall vary from country to country but in Nigeria where Boophilus species constitute about 35% of tick population (Dipeolu 1983) and where they are widespread in all ecological zones of the country (Dipeolu 1975), their contribution to the epidemiology of bacterial infections of livestock must be substantial. It is also possible that these bacteria, after transmission by ticks, reproduce to release toxins which supplement the tick toxins which are responsible for tick paralysis (Doube 1975, Allen 1977, Dipeolu 1976, Akinboade 1982). In addition, Macadam (1964) had incriminated ticks, especially Amblyomma variegatum as a vector of Dermatophilus congoensis and he based his assertion on circumstantial evidence of finding the ticks on the site of infection on the body of the animal. This study however gives credence to biological transmission of bacteria by ticks and, in view of the importance of D. congoensis as cause of streptothricosis, it is necessary to undertake studies on the mode of transmission especially whether it is transovarial or transstadial. This work is going on in our laboratory.

ИЗОЛЯЦИЯ БАКТЕРИИ ИЗ BOophilus DECOLORATUS
И BOophilus GEIGYI И ЕЕ ТРАНСМИССИЯ ЕТНИМ ПЕРЕНОСЧИКАМИ

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Резюме. Бактерии были изолированы из гемолимфы Boophilus decoloratus и Boophilus geigi которые присосались к продленому рогатому скоту в Нигерии. Все виды бактерий были также изолированы из ниц, которые они кладли, хотя некоторые из них отсутствовали у личинок, которые со временем выпустились. Кроме незараженных крошки были выращены некоторыми видами бактерий и незараженные, в лаборатории разведенные личинки B. decoloratus и B. geigi кормились ими, такие же виды бактерий были видосообразно изолированы из гемолимфы присосавшихся взрослых экземпляров, инь ими продуцированных и личинок выпуленных из них. Staphylococcus pyogenes и Pseudomonas aeruginosa были последовательно обнаружены в гемолимфе ниц и личинок обоих видов Boophilus и в то время как Proteus mirabilis был дополнительно обнаружен в B. geigi.

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