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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 transovarial 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, Dagliesh 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, Adegoke 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 transovarial 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 let 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 deficient (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

Species of ticks	Haemolymph			Eggs			Larvae		
	No. of engorged ticks punctured	No. of ticks positive for bacteria	Species of bacteria isolated	No. of batches cultured	No. positive for bacteria	Species of bacteria isolated	No. of larval batches cultured	No. positive for bacteria	Species of bacteria isolated
B. decoloratus	20	20	*Staph. pyogenes** (14) *Staph. aureus* (8) *Staph. albus* (6) *Pseud. aeruginosa* (6) *Esch. coli* (8)	20 (each) batch of 1000 eggs)	14	*Staph. pyogenes** (10) *Staph. aureus* (4) *Staph. albus* (2) *Pseud. aeruginosa* (5) *Esch. coli* (4)	20 (each batch of 1000 larvae)	20	*Staph. pyogenes** (6) *Staph. aureus* (4) *Pseud. aeruginosa* (8)
B. geigyi	20	20	*Staph. pyogenes* (12) *Staph. aureus* (10) *Staph. albus* (9) *Bac. subtilis* (16) *Pseud. aeruginosa* (4) *Esch. coli* (6) *Proteus mirabilis* (2)	20	16	*Staph. pyogenes* (12) *Staph. aureus* (8) *Staph. albus* (8) *Pseud. aeruginosa* (4) *Esch. coli* (6) *Prot. mirabilis* (2)	20	16	*Staph. pyogenes* (10) *Pseud. aeruginosa* (8) *Prot. mirabilis* (6)

* 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 innoculated with bacteria and from the eggs they produced and larvae which hatched from them

Species of ticks	Haemolymph			Eggs			Larvae		
	No. of engorged ticks punctured	No. positive for bacteria	Species of bacteria isolated	No. of egg batches cultured	No. positive for bacteria	Species of bacteria isolated	No. of larval batches cultured	No. positive for bacteria	Species of bacteria isolated
B. decoloratus	60	60	*Staph. pyogenes* (53) *Staph. aureus* (27) *Staph. albus* (32) *Pseud. aeruginosa* (51) *Esch. coli* (18)	60	51	*Staph. pyogenes* (48) *Staph. aureus* (12) *Staph. albus* (21) *Pseud. aeruginosa* (44) *Esch. coli* (9)	60	46	*Staph. pyogenes* (42) *Staph. albus* (17) *Pseud. aeruginosa* (40)
CONTROL (Rabbits without bacteria innoculation)	40	0	—	40	0	—	40	0	
B. qeigyi	60	60	*Staph. pyogenes* (58) *Staph. aureus* (54) *Staph. albus* (39) *Bac. subtilis* (14) *Pseud. aeruginosa* (47) *Pseud. aeruginosa* (47) *Esch. coli* (38) *Prot. mirabilis* (52)	60	54	*Staph. pyogenes* (54) *Staph. aureus* (19) *Staph. albus* (23) *Pseud. aeruginosa* (36) *Prot. mirabilis* (39)	60	53	*Staph. pyogenes* (50) *Prot. mirabilis* (34) *Pseud. aeruginosa* (30)
CONTROL (Rabbits without bacteria innoculation)	40	0	—	40	0	—	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 peritone ally 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 12×10^7 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 innoculated rabbits were used for *B. decoloratus* and another three innoculated ones were used for *B. geiayi*. Two rabbits without any bacterial innoculation 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 innoculated 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 innoculated 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 transovarially 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 innoculated rabbits.

The observations made from this investigation had 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 congolensis* 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. congolensis* 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 БАКТЕРИЙ ИЗ *BOOPHILUS DECOLORATUS* И *BOOPHILUS GEIGYI* И ИХ ТРАНСМИССИЯ ЕТИМИ ПЕРЕНОСЧИКАМИ

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Резюме. Бактерии были изолированы из гемолимфы *Boophilus decoloratus* и *Boophilus geigyi* которые присосались к продажному рогатому скоту в Нигерии. Все роды бактерий были также изолированы из яиц, которые они клали, хотя некоторые из них отсутствовали у личинок, которые со временем вылупились. Когда незараженные кролики были инокулированы некоторыми родами бактерий и незараженные, в лаборатории разведенные личинки *B. decoloratus* и *B. geigyi* кормились ими, такие же роды бактерий были впоследствии изолированы из гемолимфы присосавшихся взрослых экземпляров, яиц ими продуцированных и личинок вылупленных из них. *Staphylococcus pyogenes* и *Pseudomonas aeruginosa* были последовательно обнаружены в гемолимфе яиц и личинок обоих видов *Boophilus* в то время как *Proteus mirabilis* был дополнительно обнаружен в *B. geigyi*.

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