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Transmission of Bacteria Isolates Through all the Developmental Stages of Dog Ticks (Bacteriological Evidence)

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Abstract: Two species of bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) were isolated from the haemolymph of engorged dog ticks (*Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi*). The same species of bacteria were also isolated from the eggs laid by these tick species. Clean rabbits were inoculated with the same species of bacteria and clean laboratory bred larvae were fed on them to adult stage. The same species of bacteria were isolated from the haemolymph of the engorged adult females, the eggs they produced and the larvae that hatched out. *Staph. aureus* was constantly encountered in the haemolymph of adults, eggs and larvae of both *R. sanguineus* and *H. leachi leachi* while *Staph. epidermidis* was additionally consistent in *R. sanguineus*. The clinical importance of transmission of pathogenic bacteria to dogs are discussed.

Key words: Bacteria, transmission, tick, haemolymph, egg and larvae

INTRODUCTION

Arthropods carry thousands of bacterial symbionts as parasites, mutualistic partners, or protocooperants (Cazemier *et al*., 1997; Groombridge, 1992; Ishikawa, 1989). This understanding prompted several studies on the bacteria of ectoparasites; especially ticks, with the purpose of characterizing those that are of medical and veterinary importance.

Ticks play an important role in human and veterinary medicine, in particular due to their ability to transmit a wide spectrum of pathogenic micro-organisms of protozoal, rickettsial, bacterial and viral origin (Sparagano *et al*., 1999).

Bacteria of genera *Escherichia*, *Staphylococcus*, *Proteus*, *Pseudomonas* and *Enterobacter* were isolated from ticks of genera *Rhipicephalus*, *Haemaphysalis* and *Boophilus* (Rahman and Rahman, 1980). Three genera of bacteria (*Pseudomonas*, *Escherichia* and *Staphylococcus*) were also isolated from adults, eggs and larvae of *Boophilus decolaratus* and *Boophilus geigyi* (Amoo *et al*., 1987). Septicemia due to *Staphylococcus pyogenes* in rabbits fed with larval progenies of *Boophilus* and Hyalomma species has also been reported (Adegoke *et al*., 1981). A study on the occurrence of gram-negative bacteria in ticks also provided evidence to show that *Ixodes ricinus* is a potential vector of *Pasteurella* (Stojek and Dutkiewicz, 2004).

However, scanty information exists on the microbe partners of *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi* (Dog tick), which are known vectors of *Babesia canis and Babesia gibsoni* (Kuttler, 1988) *which* represent significant health hazard for domestic animals.

The aim of this work is to present data on natural bacteria isolated from *R. sanguineus* and *H. leachi leachi*, tracing the cultured bacteria through the developmental stages of the ticks.

MATERIALS AND METHODS

Collection of ticks: Adult ticks used in this study were collected from dogs brought to veterinary clinics and household dogs in Ibadan between June 2005 and May 2006. The ticks were collected using sterile forceps into sterile bijou bottles and taken to the Veterinary Microbiology and Parasitology laboratory at the University of Ibadan for identification into *Rhipicephalus sanguineus* and *Haemaphysalis leachi leachi*. After identification of ticks, each of *R. sanguineus* and *H. leachi leachi* were wiped clean with cotton wool soaked in 95% alcohol and each of the ticks was kept in individual well labeled sterile bijou bottle. The study then proceeded in stages.

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Stage I: Each of the ticks was punctured with a fine aseptic dissecting pin for haemohymph on the abdomen near the fourth leg. With the aid of a sterile platinum wire loop, a small inoculum of the haemolymph was streaked on blood agar and incubated for 24-48 h at 37°C.

The ticks were returned into individual bijou bottle and incubated in the acaridium at 25°C and 85% relative humidity (R.H) and 12 h of light and 12 h of darkness photo-periodic conditions for oviposition. Identification of bacterial isolates was based on morphological, cultural and biochemical characteristics as described by Cowan and Steel (1966).

Stage II: At the end of oviposition of the engorged ticks punctured for haemolymph in stage I, 0.03 g of eggs equivalent to 657 eggs (Adejinmi, 2005); from the egg mass of each tick were crushed in 2 mL of sterile normal saline with sterile mortar and pestle. Using a sterile platinum wireloop a small innoculum was streaked on blood agar and incubated for 24-48 h at 37°C. Identification of bacteria followed as in stage I.

The remaining egg masses from each tick were returned into the acaridium for further incubation.

Stage III: About 500 larvae 3-5 days old which hatched out from each tick egg mass part of which was crushed in stage II were taken and crushed in 2 mL of sterile normal saline with sterile mortar and pestle and cultured in blood agar as in stages I and II.

Transmission experiment: Six New Zealand white rabbits which had not been exposed to tick infestation were used for this study. Total 2 rabbits each were used for *R. sanguineus* and *H. leachi leachi* and 2 rabbits served as controls. All the rabbits were screened for bacteria in their blood before infestation with ticks. Ticks whose haemolymph did not show bacterial growth and whose part of the eggs when cultured did not show bacterial growth on blood agar were used for this study. The larvae from these ticks were used to infest rabbits using the ear bag method (Inokuma *et al*., 1997). On day 12 after tick attachment, 2 rabbits were injected peritoneally with 1 mL of normal saline bacterial suspension which contain about 4×10^7 bacteria/mL of suspension from *R. sanguineus*. The bacterial suspension was obtained by pooling all the larvae which carried bacterial infection as indicated by the results of haemolymph puncture of adults which produced their eggs (stage I) and eggs and larval cultures (stage II and III). The bacterial solution was then standardized by the method of Miles and Misra (1938).

Inoculation of bacterial suspension was made on the 12th day of tick infestation because preliminary observations in our laboratory showed that nymph of *R. sanguineus* and *H. leachi leachi* start to engorge on rabbit around this period. The larvae were allowed to molt into nymphs, fed again on the rabbits as nymphs and adults.

Twenty Engorged ticks were carefully detached with sterile forceps from days 28-30. Isolation of bacteria from the haemolymph of the adult and the eggs they laid and the larvae which hatched from them were as carried out as described in stages I, II and III.

Two inoculated rabbits were used for *R. sanguineus* and another 2 inoculated ones used for *H. leachi leachi.* Two rabbits that served as control had no bacterial suspension injected into them.

RESULTS

Two species of bacteria, *Staphylococcus aureus* and *S. epidermidis*, were isolated from the haemolymph of *R. sanguineus* adults, eggs and larvae while only one species, *Staphylococcus aureus*, was isolated from the haemolymph of *Haemaphysalis leachi leachi* adults, eggs and larvae (Table 1).

The same species of bacteria were isolated from haemolymph, eggs and larvae of ticks that engorged on rabbits as from the ticks which fed on dogs. The species of bacteria were consistent in all the stages of *R. sanguineus* and *H. leachi leachi.* They were also consistent in all the stages of ticks that fed on experimental rabbits. No bacteria were isolated from all stages of ticks that fed on the control rabbits (Table 2).

Table 1: Bacteria Isolated from the haemolymph of adult *R. sanguineus* and *H. leachi leachi* collected from dogs, the eggs they produced and the larvae which hatched from them

	Haemolymph			Eggs			Larvae		
	No. of engorged ticks punctured	No. +ve for bacteria	Species of Bacteria from the total ticks	No. of Egg hatched	No. +ve for bacteria	Specie of bacteria	No. of Larvae batches	No. +ve for bacteria	Specie of bacteria
R. sanguineus	30	30	*Staph. aureus* (18) *Staph. epidermis* (12)	30	24	*Staph. aureus* (18) *Staph. epidermis* (6)	30	18	*Staph. aureus* (12) Staph. (6) *epidermis*
H. leachi leachi	30	24	*Staph. aureus* (24)	30	17	*Staph. aureus* (17)	30	12	*Staph. aureus* 12

Table 2: Bacteria Isolated from the haemolymph of adult *R. sanguineus* and *H. leachi leachi* which had engorged on rabbits inoculated with bacteria and from the eggs they produced and larvae which hatched from them

	Haemolymph			Eggs			Larvae		
	No. of engorged ticks punctured	No. +ve for bacteria	Species of bacteria from the total ticks	No. of Egg hatched	No. +ve for bacteria	Specie of bacteria	No. of Larvae batches	No. +ve for bacteria	Specie of bacteria
R. sanguineus	20	20	*Staph.* (16) *aureus* *Staph.* (4) *epidermis*	20	14	*Staph.* (10) *aureus* Staph. (4) *epidermis*	20	8	*Staph.* (4) *aureus* *Staph.* (4) *epidermis*
Control	20	0		20	-		20	0	
H. leachi leachi	20	14	*Staph.* (14) *aureus*	20	10	*Staph.* (10) *aureus*	20	8	*Staph.* (8) *aureus*
Control	20	0	-	20	0	-	20	0	-

DISCUSSION

Our study, in concert with previous research, provides evidence of the presence of *Staphylococcus* species in the haemolymph, larvae and eggs of ticks (Amoo *et al*., 1987); although, further work is required to understand the role they play in ticks. The isolation of *Staphylococcus aureus* and *S. epidermidis* from *R. sanguineus* and only *S. epidermidis* from *H. leachi leachi* in this work provides a clue that bacterial survival in ticks could be species specific. However it has been documented that ticks can be infected by fastidious, facultative or obligate intracellularly growing or even non-cultivable bacteria (Noda *et al*.,1997; Zhu *et al*., 1992); making the identification of the specific tick-inhabiting bacteria complicated or impossible by conventional cultivation techniques.

The inability to isolate bacteria from the haemolymph, eggs and larval extract of ticks that fed on control rabbits but only from ticks that fed on dogs and experimental rabbits suggests that ticks get infected with *Staphylococcus auerus* and *S. epidermidis* when they feed and could maintain the organisms through all the developmental stages. This also implies that these isolated bacteria may not be normal flora of ticks as suggested by some previous works (Noda *et al*., 1997; Zhu *et al*., 1992).

The maintenance and transmission of bacteria by all stages of ticks could complicate the clinical picture presented by tick infestation on animals, particularly dogs, since it may imply that the pyrexia noticed as a result of hemoparasitism (e.g., as in canine babesiosis) may also have some level of contribution by bacteria harbored in the ticks. The tendency to also worsen the problem in the dogs by the release of bacterial toxins that could lead to septicemia also exists in such clinical cases.

In order to further ascertain the significance of our study, a follow-up study using molecular methods to confirm the suggested association of *Staphylococcus* sp. with the dog ticks is at a planning stage in our laboratory. The proposed investigation will be based on the utilization of a broad range and genus/species-specific polymerase chain reaction (PCR) primers that targets highly conserved regions of genes encoding for 16S rDNA of bacteria (Head *et al*., 1998; Sparagano *et al*., 1999), thereby enabling the detection and fingerprinting of the bacteria isolated in the present study along with other rare or unexpected bacteria. The approach will also overcome the limiting factors of culture-based methods. The inability to cultivate more bacterial species could be due to the limitations of our cultivation systems. However the results of this study indicate a potential risk of staphylococcosis maintenance and transmission by ticks which consequently may complicate the clinical picture of the diseases transmitted by these ticks.

This study is one of the few reports of transovarian transmission of bacteria by ticks, the implication of which may vary from one country to another. In Nigeria, *R. sanguineus* and *H. leachi leachi* constitute 99% of dog ticks and are widespread in all ecological zones of the country (Dipeolu, 1975; Adejinmi, 2005). Hence, their contribution to the epidemiology of bacterial infections of dogs could be significant.

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