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STUDIES ON BACTERIAL ASSOCIATION WITH *AMBLYOMMA* *VARIEGATUM* (FABRICIUS, 1974) (ACARINA : IXODIDAE)

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Amblyomma variegatum, a haematophagous ectoparasitic arthropod, is a known vector of several protozoan, bacterial, rickettsial and viral parasites of livestock. Being a 3-host tick, the larvae, nymphs and adults are active in transmitting these microorganisms. In this study, four bacteria *Staphylococcus pyogenes*, *Enterobacter alvei*, *Escherichia coli* and *Klebsiella rhinoscleromatis* were isolated from the haemolymph of *A. variegatum* detached from trade cattle. *Staphylococcus pyogenes* and *Klebsiella rhinoscleromatis* were found in the egg and larval extracts. It was observed that these two bacteria are transmitted transovarially by *A. variegatum*.

MATERIAL AND METHOD

The ticks used for this experiment were collected from cattle stationed at the Veterinary Control Post, Bodija, Ibadan. Only ticks of the genus *Amblyomma variegatum* were collected and taken to the laboratory. The ticks were carefully weighed in the laboratory using Mettler Analytical Balance H15 and only those that attained the weight of one gram were used for this experiment. Twenty of such ticks were used. They were washed several times with sterile water and finally wiped with cotton wool immersed in seventy percent (70%) alcohol. Each tick was placed individually in labelled and sterile universal bottles and kept at room temperature of 25°C (temperature varying between 24°C and 32°C) and relative humidity of 85%. The experiment was carried out in phases.

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Each tick had its fourth leg amputated and a puncture made at the junction between the leg stump and the body of the tick with a sterile dissecting pin for haemolymph which was taken with the aid of a sterile platinum loop.

The haemolymph so released was used to inoculate blood agar (BA) and Mackonkey agar plates. The ticks were labelled A-T (20 ticks) and for each sample 2 plates were inoculated, one on blood agar (BA) and the other on Mackonkey agar. The agar media were incubated in air at 37°C for 18—24 hrs. The ticks were then incubated in the laboratory at 25°C and 85% relative humidity. Identification of bacteria isolates were based on their morphological, mode of culture, and biochemical characteristics as described by Cowan and Steel (1966).

At the end of oviposition of the ticks (A-T) punctured for haemolymph, about 200 eggs were rolled out into a sterile mortar and pestle and crushed in a few drops of normal saline solution. With the aid of a sterile platinum loop, the egg solution was inoculated on blood agar and Mackonkey agar. Identification of bacteria isolated is as described before. The remaining eggs from each tick were returned to the dessicator and incubated at 25°C and relative humidity of 85% R.H. until they hatched into larvae.

About 200 larvae hatched from the eggs were crushed in a sterile mortar and pestle and cultured in blood agar and Mackonkey agar using a sterile platinum loop. The agar media with the cultures were incubated in air at 37°C for 18—24 hrs.

About 200 larvae from phase 2 were fed on rabbits ears using the cloth bags. Ten rabbits (6 months old) which had been previously unexposed to tick infestation were used for this experiment. Before use, the rabbits were screened for the presence of bacteria in their blood. Drops of blood obtained from their ear veins were cultured in Agar to determine if there were bacteria. After feeding on the rabbits ears for 7 days, the engorged larvae were removed from the rabbits ears. On the 14th day after infestation, drops of blood obtained by pricking the ear veins of the rabbits were cultured in blood agar and Mackonkey agar as in the previous experiments.

RESULTS AND DISCUSSIONS

Table 1 shows the result of this experiment. Four genera of bacteria, *Staphylococcus pyogenes*, *Klebsiella rhinoscleromatis*, *Enterobacter alvei* and *Escherichia coli* were isolated from the haemolymph of *Amblyomma variegatum*. Of these bacteria, only two genera, *S. pyogenes* and *K. rhinoscleromatis* were found in the egg and larval extracts of *A. variegatum*. It was observed that four of the punctured engorged

Table 1

Bacteria isolated from the haemolymph, eggs and larvae of
A. variegatum collected from trade cattle in Ibadan

Species of tick	HAEMOLYMPH			EGGS (20 eggs)			LARVAE (20 larvae)		
	Number		Species of bacteria isolated from cumulative number of ticks	Number		Species of bacteria isolated from cumulative number of ticks	Number		Species of bacteria isolated from cumulative number of ticks
	of engorged ticks punctured	positive for bacteria		of egg batches cultured	positive for bacteria		larval batches cultured	positive for bacteria	
<i>A. variegatum</i>	20 Specimens A—T	20 Specimens A—T	<i>Staphylococcus pyogenes</i> (18) <i>Klebsiella rhinoscleromatis</i> (12) <i>Enterobacter alvei</i> (6) <i>Escherichia coli</i> (6)	16 4 females died before laying eggs	16	<i>Staphylococcus pyogenes</i> (15) <i>Klebsiella rhinoscleromatis</i> (10)	16	16	<i>Staphylococcus pyogenes</i> (15) <i>Klebsiella rhinoscleromatis</i> (10)

females died before egg-laying. The rabbits on which the larvae had fed, produced septicaemia with only *S. pyogenes* and *K. rhinoscleromatis*. No bacteria were isolated from the haemolymph, eggs and larval extracts of ticks that engorged on control rabbits. It was noted that only *Staphylococcus pyogenes* and *Klebsiella rhinoscleromatis* were encountered in the haemolymph, eggs and larvae of *A. variegatum*.

Of all the bacteria isolated from the haemolymphs, eggs and larvae of *A. variegatum*, only *Staphylococcus pyogenes* and *Klebsiella rhinoscleromatis* were consistently found. They were also the only ones that caused septicaemia in rabbits through inoculation. These two bacteria can therefore be said to be transmitted transovarially by *A. variegatum*.

Transovarial transmission for these bacteria could be followed from the adult haemolymph through the eggs to the larvae of *A. variegatum*. While four genera of bacteria were found in the haemolymph of adult *A. variegatum*, two, *Enterobacter alvei* and *Escherichia coli* were not found in the egg and larval extracts. This indicates that these two bacteria are not transmitted transovarially in *A. variegatum*.

Adegoke et al., (1981) observed that the haemolymph and the oviposited eggs of clean laboratory-bred ticks did not show bacterial growth. Therefore, the bacteria thus isolated from this experiment must have their source from the trade cattle on which the ticks had fed. The fact that no bacteria were isolated from the haemolymph, eggs and larval extracts of ticks that engorged on control rabbits but only from ticks that fed on trade cattle means that these bacteria isolates do not normally form part of the microbial flora of the haemolymph, eggs and larvae of *A. variegatum*.

Observations made from these results open a new avenue to the knowledge of the transmission of pathogenic bacteria amongst livestock in Nigeria. It might be possible that some diseases like paralysis could be caused by toxins produced by some of these bacteria, as reported by *Doube and Kemp* (1975), *Dipeolu and Ogunji* (1977) and *Akinboade* (1982).

Rahman and Rahman (1980) had isolated bacteria of the genera *Staphylococcus*, *Escherichia*, *Pseudomonas*, *Proteus* and *Enterobacter* from ticks of the genera *Boophilus*, *Rhipicephalus* and *Haemaphysalis*. They also observed that the areas where the ticks had bitten the forest workers showed slight inflammation. *Adegoke et al.*, (1981) also reported septicaemia with *Staphylococcus pyogenes* in rabbits fed with larvae of *Boophilus* and *Hyalomma*.

Macadam (1964) had incriminated *Amblyomma variegatum* as a vector of *Dermatophilus congolensis*, a bacterium that causes bovine streptothricosis. It is confirmed from this result, however, that bacteria are transmitted by ticks. The mode of transmission of *D. congolensis* by *Amblyomma variegatum* is not yet known. *Okorie* (1976) isolated the Conga and Dugbe virus from *A. variegatum*. It is possible therefore that *A. variegatum* is capable of transmitting many pathogenic microorganisms in this country.

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ETUDES CONCERNANT L'ASSOCIATION BACTÉRIENNE AVEC AMBLYOMMA VARIEGATUM (FABRICIUS, 1794) (ACARINA : IXODIDAE)

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Résumé

L'arthropode ectoparasitaire hématophage *Amblyomma variegatum* est un vecteur connu pour plusieurs protozoaires et parasites bactériens, rickettsiens et viraux présents dans les effectifs d'animaux. Etant un parasite à trois hôtes, les larves, les nymphes et les formes adultes représentent des transmetteurs actifs de ces microorganismes. Dans le cadre de l'étude décrit plus haut quatre bactéries, *Staphylococcus pyogenes*, *Enterobacter alvei*, *Escherichia coli* et *Klebsiella rhinoscleromatis* ont été isolées de la hémolymphes de l'arthropode *A. Variegatum* récolté à partir des bovins commerciaux, *Staphylococcus pyogenes* et *Klebsiella rhinoscleromatis* étaient présentes dans l'oeuf et dans les extraits larvaires. On a constaté que ces deux bactéries sont transmises par voie transovarienne par *A. variegatum*.

STUDIEN ÜBER BACTERIELLE ASSOZIATION MIT AMBLYOMMA
VARIEGATUM (FABRICIUS, 1794) (ACARINA : IXODIDAE)

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Zusammenfassung

Die hematophagen und ectoparasiten *Amblyoma variegatum* sind bekannte Vektoren für mehrere Protozoen und bakteriellen rickettiellen und viralen Parasiten die in Tierbestände anwesend sind. Sie sind Parasiten mit drei Logis, die Larven, die Nymphen und die Erwachsenen Alle drei Formen sind aktive Überträger dieser Mikroorganismen.

Während des Studiums, vier Bakterien, und zwar *Staphylococcus pyogenes*, *Enterobacter alvei*, *Escherichia coli* und *Klebsiella rhinoscleromatis* sind ausdem Hemolymph der Gliederfüßler *A. variegatum* isoliert worden. Die Gliederfüßler sind von gewöhnlichen Rinder entnommen. *Staphylococcus pyogenes* und *Klebsiella rhinoscleromatis* waren anwesend in Ei und in dem Extrakt aus Larven.

Es ist festgestellt dass diese zwei Bakterien auf transovariumweise übertragen werden. Die Übertragung ist von den Gliederfüßlern *A. variegatum* durchgeführt.

ИСПЫТАНИЯ КАСАЮЩИЕ БАКТЕРИАЛЬНОЙ АССОЦИАЦИИ
С. AMBLYOMMA VARIEGATUM (FABRICIUS, 1794) (ACARINA : IXODIDAE)

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Резюме

Кровососущий эктопаразитарный артропод *Amblyomma variegatum* является известным вектором большого количества протозоа и бактериальных паразитов, рикетсий и вирусов существующих в поголовиях животных. Будучи паразитом с тремя видами хозяинов, личинки, нимфы и взрослые формы являются активными переносителями этих микроорганизмов. В рамках нижеописанной научной работы четыре бактерии а именно *Staphylococcus pyogenes*, *Enterobacter alvei*, *Escherichia coli* и *Klebsiella rhinoscleromatis* были изолированы из гемолимфы артропода *A. variegatum* взятым от коммерческого крупного рогатого скота, *Staphylococcus pyogenes* и *Klebsiella rhinoscleromatis* существовали в яйце и личиночных экстрактах. Было установлено что обе бактерий переданы путём яичника от *A. variegatum*.

L'arthropode ectoparasitaire hématophage *Amblyomma variegatum* est un vecteur connu pour plusieurs protozoaires et parasites bactériens rickettiens et viraux présents dans les effectifs d'animaux. Étant un parasite à trois hôtes, les larves, les nymphes et les formes adultes représentent des transmetteurs actifs de ces microorganismes. Dans le cadre de l'étude décrite plus haut quatre bactéries (*Staphylococcus pyogenes*, *Enterobacter alvei*, *Escherichia coli* et *Klebsiella rhinoscleromatis*) ont été isolées de la remonte de l'arthropode *A. variegatum* récolté à partir des bovins commerciaux. *Staphylococcus pyogenes* et *Klebsiella rhinoscleromatis* étaient présents dans l'œuf et dans les extraits larvaires. On a constaté que ces deux bactéries sont transmises par voie transovarienne par *A. variegatum*.

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