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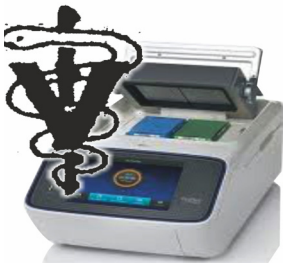

BACTERIOLOGICAL EVALUATION OF POOLED GUT CONTENTS OF *AMBLYOMMA* SPECIES INFESTING GOATS IN MAIDUGURI, BORNO STATE, NIGERIA

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Abstract

The culture, isolation, identification and biochemical characterization of bacteria from homogenized gut contents of Amblyomma ticks feeding on goats in Maiduguri were conducted in this study. A total of thirty-five (35) isolates were obtained including Escherichia coli and Staphylococcus aureus with the highest frequency of 10 (28.6%) each, Proteus mirabilis, Enterococcus spp. and Bacillus spp. with 4 (11.4%) each and Corynebacterium spp. with 3 (8.6%). Upon characterization, Escherichia coli appeared greyish-white with large, thick, moist, smooth, opaque or mucoid on MacConkey agar and as pinkish, rod or bacilli, gram negative, indole, nitrate reduction and MR-VP positive but oxidase negative. Staphylococcus aureus appeared fairly large golden-yellow, round, opaque and as purple-violet colour, spherical or cocci in cluster, gram positive, catalase, glucose and coagulase positive but oxidase negative. Proteus mirabilis appeared swarming with characteristic smell on MacConkey agar, slender rods, gram negative, indole, citrate, lactose, sucrose, mannitol, and generally urease positive. Enterococcus Species appeared mucoid on MacConkey agar and in pairs or chain with rod shape, gram positive and lactic acid-fermenting. Bacillus species appeared as circular ridges, smooth, moist, sticky and medium sized, arranged in long chain, rod, gram positive, and catalase positive. Corynebacterium species were small, grayish, granular, mostly translucent with opaque centers, convex with continuous borders, rod or bacilli and showed metachromatic granules when stained with methylene blue, gram positive, catalase positive but some are oxidase negative. Bacterial isolates found in the homogenized midgut of Amblyomma ticks were Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Enterococcus Species, Bacillus species and Corynebacterium species.

Keywords: Amblyomma, Goats, Gut content, Bacteria, Culture, Characterization

Introduction

Ticks are vectors that are of significant importance in the transmission of various pathogens, including bacteria, viruses and protozoa, which cause diverse diseases such as anaplasmosis, Lyme disease, tick-borne encephalitis (TBE) and others[1]. Tick infestations also result in economic losses from reduced productivity, veterinary costs and acaricide use [2][3]; the presence of ectoparasites in goat production is critically associated with substantial losses in livestock production worldwide [4]. Ticks of the genus *Amblyomma* are hard-bodied ectoparasites belonging to the family, Ixodidae. They are widely distributed across tropical and subtropical regions of the world and known for their medical and veterinary importance due to their role as vectors of numerous pathogens. *Amblyomma* species infest a wide variety of hosts, including reptiles, birds and mammals, and are particularly noted for their aggressive feeding behavior and long attachment periods [5].

Globally, over 130 species of *Amblyomma* have been described, with significant presence in Africa, South and Central America and parts of Asia [6]. These ticks are competent vectors of several bacterial and protozoan pathogens, including *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever in the Americas and *Ehrlichia ruminantium*, the cause of heartwater disease in ruminants in Africa and the Caribbean [7].

In Nigeria, *Amblyomma variegatum* is the most commonly reported species, often referred to as the tropical bont tick. It is of major economic importance due to its role in transmitting *Ehrlichia ruminantium* to cattle, leading to substantial losses in livestock productivity [8]. This tick is also known to cause severe skin lesions that can predispose animals to secondary infections and reduce the quality of hides and skins [9]. Other *Amblyomma* species such as *A. lepidum* and *A. hebraeum* have also been occasionally reported in some regions of Nigeria, particularly in border areas or through livestock trade routes.

Surveillance studies in Nigeria have underscored the prevalence and seasonal distribution of *Amblyomma* ticks, highlighting the impact of climatic and ecological factors on their population dynamics [10]. With changing environmental conditions and increased livestock movements, the risk of *Amblyomma*-borne diseases is expected to grow, necessitating continuous monitoring and integrated tick control strategies.

The Food and Agriculture Organization (FAO) also notes the economic importance of small ruminants, including goats, in sub-Saharan Africa. In regions like Northern Nigeria, small ruminants are more commonly owned than cattle, providing meat, milk, and income, especially for low-income households [11] (FAO, 2012). Bacterial culture and isolation are essential as they have long been accepted as the gold standard for laboratory confirmation of bacterial infections [12].

Materials and Methods

Description of Study Area

This study was conducted in Maiduguri, the capital city of Borno state, located in the Sahel Savannah zone of Northern Nigeria, between latitude 11°50’8.91N and longitude 13°9’25.6°E. The climate of Maiduguri is characterized by a long period of dry season for the rest of the year [13] (Hess *et al.,* 1995).

Collection of Ticks

Visits were made to the livestock market in Maiduguri which is popularly known as the Kasuwan Shanu once a week, for the period of four weeks starting from the first week February, 2022 to the last week of the Month. During these visiting periods, ticks were collected in pools of 10 making a total of forty, while the goats were physically examined and ticks found on them, handpicked into universal container.

Ticks were collected from different parts of the body using forceps and hand gloves. When required, small hairbrush chipped in ethanol was used for the collection of ticks. Adequate precautions were taken to preserve the mouthparts and some appendages of the ticks during collection to help in the identification. Collected ticks were put into clean, properly labeled vials containing 70% alcohol or 5% glycerol for preservation. The vials were immediately transported to the Department of Veterinary Parasitology and Entomology Laboratory of the University of

Maiduguri for further identification and analyses.

Identification of Ticks

The identification of ticks was done with the aid of Petri dish, forceps, filter paper and stereomicroscope.

The ticks in the containers were transferred to a petri dish, spread onto filter paper to absorb excess preservatives and examined under stereomicroscope for identification using standard identification keys as described by walker *et al*. [14].

Preparation of Tick Homogenate in the Laboratory

Ticks were washed in distilled water, then in 70% alcohol to rinse further and immobilized before a thorough rinsing in normal saline. Homogenization of the ticks, using a sterile mortar and pestle following washing, resulted in their fragmentation, and facilitated the yield of homogenates.

Inoculation of Ticks Homogenates on Media

The media used for the purpose of this research were blood and the MacConkey Agar respectively; homogenates, were then inoculated on the media. These are two different differential media used to cultivate micro-organisms., the blood agar is enriched with the blood nutrients and it provides enough nutrients in which most microorganisms utilize for growth. While MacConkey agar is used to select gram negative bacteria from non-fermenting bacteria colonies respectively. So, in this research, blood agar was first used to culture a lot of species before MacConkey agar was introduced for the isolation of the organisms.

Incubation for Culture Growth

The inoculated media was kept in the incubator for eighteen (18) to twenty-four (24) hours for observation of growth. However, in most cases if no growth was obtained, duration of incubation was extended for forty-eight (48) hours so as to ensure full lap count and then results were being taken from the media. However, even if no growth was being observed the result was reported as no growth.

Biochemical Testing of Bacterial Isolates

Catalase Test conducted following a standard procedure

Tube Method

- One to two milliliters (1–2 ml) of hydrogen peroxide solution was poured into a test tube.
- A sterile wooden stick or a glass rod was used to take several colonies of the 18 to 24 hours test organism and immersed in the hydrogen peroxide solution.
- Observation was made for immediate bubbling.

Slide Method

- A loop or sterile wooden stick was used to transfer a small amount of colony growth on the surface of a clean, dry glass slide.
- A drop of 3% H_2O_2 was placed on the glass slide
- Observation was made for the evolution of oxygen bubbles.
- In Positive result, copious bubbles would be produced which will be active and bubbling e.g., staphylococci
- Negative result – Absence or very few bubbles will be produced e.g., streptococcus and enterococcus species

Citrate Test

- A test tube containing citrate medium was incubated with a small amount of bacteria.
- It was incubated at 30 – 37^{0}C during 24–48 hours.
- Positive test result – showed growth in citrate medium or growth colour change to blue
- Negative test result – No growth in citrate medium and no change in color.

Indole Test

- Some drops of peptone water were poured in a test tube.
- Colonies of bacteria were packed using wire loop dipped carefully into the test tube containing peptone water.
- Cotton wool was used to cover top of the test tubes and incubated for 24 hours
- Kovais reagent was pipetted out and 2–3 drops were poured into the test-tube after incubating.
- Test is positive if no colour change is observed, but negative when no organism is capable for producing tryptophane.

Urease Test

- Suspend one colony from the pure culture, which is to be investigated in VP/MR medium.
- Incubate at 30 – 37°c during 24 – 48 hours
- Add 0.2ml of 40% kots and then 0.6ml of alpha nephtol.

Result

Positive result – The colour changes to pink

Negative result – No colour change

Nitrate Reduction Test

- Inoculate nitrate growth with a heavy growth of test organism using an aseptic technique
- Incubate at an appropriate temperature for 24 to 48hrs
- Add one dropper full of sulfa nitic acid and one dropper full of an a-naplhylamine to each broth
- At this point, a colour change to red indicates a positive nitrate reduction test. If you get a red colour, then you stop at the point.

Oxidase Test

Direct Plate method

- Add 2–3 drops of reagent directly to suspect colonies on an agar plate. Do not flood the plate with reagent.
- Observe for color change within 10 seconds. The direct plate method should be carried out on non-selective agar plate.

Results

The results of the bacterial isolates from homogenized midgut of *Amblyomma* ticks of goats are shown in Table 4.1. A total of thirty-five (35) isolates were obtained including *Escherichia coli* and *Staphylococcus aureus* with the highest frequency of 10 (28.6%) each, *Proteus mirabilis, Enterococcus* spp. and *Bacillus* spp. with 4 (11.4%) each and *Corynebacterium* spp. with 3 (8.6%).

Table I: Bacterial Isolates from Homogenized Midgut of *Amblyomma* Ticks on Goats

Bacteria	Frequency (%)
Escherichia coli	10 (28.6)
Proteus mirabilis	4 (11.4)
Staphylococcus aureus	10 (28.6)
Enterococcus Spp.	4 (11.4)
Bacillus Spp.	4 (11.4)
Corynebacterium Spp.	3 (8.6)
Total Isolates	35(100)

Table II: Descriptive Analyses of the Bacterial Isolates

Bacterial isolate	**Colonial appearance**	**Microscopic appearance**	**Staining Characteristics**	**Biochemical characteristics**
Escherichia coli	large, thick, greyish white, moist, smooth, opaque or mucoid colonies on MacConkey	pink rod shape to bacilli	Gram-negative	Indole, MR-VP broth and Nitrate reduction test positive, oxidase negative
Proteus mirabilis	Swarming	slender rods	Gram-negative	Indole, citrate, lactose, maltose, sucrose, mannitol, urease positive
Staphylococcus aureus	Fairly large golden yellow round and opaque	purple, cocci in cluster	Gram-positive	Glucose fermenters, oxidase negative coagulase and catalase positive
Enterococcus species	mucoid colonies on MacConkey	pairs or chain with rod shape	Gram-positive	Lactic acid fermenters
Bacillus species	circular ridges, smooth, moist, sticky and medium sized colonies	long chain and rod in shape	Gram positive	Catalase positive

Discussion

Bacterial isolates from homogenized midgut of *Amblyomma* ticks of goats in this study revealed *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis, Enterococcus* spp., *Bacillus* spp. and *Corynebacterium* spp. The results were in tandem with the findings of Loong *et al.*, (2020) [15] who also isolated most of these extracellular bacteria in Ixodid ticks from farm animals and rodents from cultural isolates. Ixodid ticks have been documented to play an important role in the transmission of a variety of zoonoses of bacterial, viral and protozoan origins while also harboring a reservoir of microorganisms [16]. Hard ticks feed on mammals for several days and so have the opportunity to ingest microbes. The gut of tick is an important organ in terms of blood digestion, absorption, excretion and pathogens migration, invasion and development [17]. *Escherichia coli* is one of the two predominant isolates from this study; most of these goats kept in Maiduguri are kept under poor hygienic environment and survive as scavengers in environments littered with flies. These flies could transmit goats or human gut bacteria by smearing them on the skin of other animals whereby they end up being taken up by ticks during feeding. Thus, its presence in ticks suggests a potential for environmental acquisition or transmission across hosts.

Tick-uptake of bacterial organism from the environment facilitate their survival in the vector host in which some of them are able to replicate within the parasite [18]. Biu *et. al*. (2015) [19] had earlier isolated *Streptococcus*, *Staphylococcus*, *Corynebacterium* and *Proteus* species from *Hyalomma*, *Amblyomma* and *Rhipicephalus* ticks in Maiduguri. Bacteria from the genera Staphylococcus, Bacillus, Corynebacterium, Proteus, Enterococcus and Escherichia isolated in this study, were reported across many tick species [20];[21], suggesting that these bacteria may have a role in the physio-biology of the tick or in transmission of tick pathogens.

Tick as obligatory blood sucking arthropods are important vectors of animal pathogens, particularly bacteria associated with tick pyemia, acute and subacute mastitis, septicemia, abscessation and lameness. Isolates from *Boophilus* included *Staphylococcus aureus*, *Proteus* and *Corynebacterium*. Isolates from *Hyalomma* were *Staphylococcus aureus* and *Corynebacterium*, those from *Amblyomma* were *Corynebacterium* while *Rhipicephalus* isolates were *Staphylococcus* and *Corynebacterium* [22].

There is close association of cattle ticks with bacteria pathogens with reports showing ticks as reservoirs of *Staphylococcus aureus* and this predisposes livestock to certain disease conditions such as abscess, septicemia, tick borne fever, tick borne typhus and complicated dermatophilosis. Staphylococcal infections among farm animals have been intensively studied, particularly on methicillin-resistant *S. aureus* and other species that cause mastitis [23]. Though, most Staphylococcus species are mainly innocuous commensals on livestock skin [23], one explanation could be that the culture methods used in this study favored pathogenic strain.

Corynebacterium causes nasal, nasopharyngeal and tonsillar diphtheria often with marked oedema of the neck, toxaemia with cardiac or neural complications.

Proteus species are implicated in urinary tract infections, abdominal and wound infections, septicemia and other systemic pathologies[24].

Amblyomma ticks are associated with severe forms of cutaneous dermatophilosis caused by the bacterium *Dermatophilus congolensis*. It has not been determined whether ticks have specific role in the perpetuation and spread of *Staph*. *aureus* or whether the role played is merely that of mechanical transmission alone [19]. Ivan (2010) [25] isolated *Enterobacteriacea* and *Bacillaceae* from *Amblyomma* species, most commonly *Pseudomonas.*

Conclusion

The identification of these bacterial species within ticks has several implications:

Disease Transmission: Ticks harboring pathogenic bacteria like *E. coli*, *Staphylococcus aureus*, *Corynebacterium* spp. and others found in this study could potentially transmit these pathogens to their hosts during feeding, and this may lead to different infections in the animal hosts.

Antibiotic Resistance: The presence of diverse bacterial communities within ticks raises concerns about the potential for antibiotic resistance gene transfer, especially if these bacteria interact with other microbial populations in the environment or within hosts.

Surveillance and Control: Understanding the microbial composition of ticks can aid in developing targeted strategies for tick control apart from the ability to monitor the spread of tick-borne diseases.

The findings of the current study do show that in addition to common intracellular tick-borne pathogens such as the rickettsial agents, other extracellular bacterial pathogens (gut content of ticks) associated with human or animal infections could also be recovered and this provide a baseline information of the culturable bacterial species in ticks of livestock such as goats. This study represents an attempt in culturing these tick-associated extracellular bacteria, which provides the opportunity for future research in determining their roles in tick biology and the transmission of pathogens, and also highlighting the importance of considering other potential pathogens carried within ticks.

References

1 Madison-Antenucci, S., Kramer, L. D., Gebhardt, L. L.s & Kauffman, E. (2020). Emerging tick-borne diseases. *Clinical Microbiology Reviews, 33*(2), e00083-18. https://doi.org/10.1128/CMR.00083-18

2 Pegram, R., Tatchell, R., De Castro, J., Chizyuka, H., Creek, M., McCosker, P., Moran, M. & Nigarura, G. (1993). Tick control: New concepts. *World Animal Review*, *74*, 2–11.

3 Kasaija, P. D., Estrada-Peña, A., Contreras, M., Kirunda, H. & de la Fuente, J. (2021). Cattle ticks and tick-borne diseases: A review of Uganda's situation. *Ticks and Tick-borne Diseases, 12*, 101756. https://doi.org/10.1016/j.ttbdis.2020.101756

4. Sonenshine, D. E. (1991). *Biology of ticks* (Vol. 1). Oxford University Press.

5. Guglielmone, A. A., Robbins, R. G., Apanaskevich, D. A., Petney, T. N., Estrada-Peña, A. & Horak, I. G. (2010). *The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: A list of valid species names*. Zootaxa, 2528, 1–28.

6. Nava, S., Guglielmone, A. A. & Estrada-Peña, A. (2009). Host ecology instead of host phylogeny drives the host specificity in the genus *Amblyomma* (Acari: Ixodidae). *International Journal for Parasitology, 39*(4), 407–416. https://doi.org/10.1016/j.ijpara.2008.09.005

7. Jongejan, F. & Uilenberg, G. (2004). The global importance of ticks. *Parasitology, 129*(S1), S3–S14. https://doi.org/10.1017/S0031182004005967

8. Kamani, J., Sannusi, A., Egwu, O. K., Dogo, G. I., Tanko, T. J., Kemza, S. Y., ... & Mbaya, A. W. (2017). Prevalence and significance of *Amblyomma variegatum* in the transmission of *Ehrlichia ruminantium* among cattle in Nigeria. *Journal of Veterinary Science & Medical Diagnosis, 6*(1), 1–5. https://doi.org/10.4172/2325-9590.1000229

9 Walker, A. R., Bouattour, A., Camicas, J. L., Estrada-Peña, A., Horak, I. G., Latif, A. A., ... & Ticks, A. (2003). *Ticks of domestic animals in Africa: A guide to identification of species*. Bioscience Reports.

10 Dipeolu, O. O., Akinboade, O. A., & Ogunji, F. O. (1992). Studies on the ecology of ticks in Nigeria: Seasonal distribution of ticks on livestock from three vegetation zones. *International Journal of Tropical Insect Science, 13*(4), 639–644. https://doi.org/10.1017/S174275840001389X

11. Food and Agriculture Organization of the United Nations (FAO). (2012). *Small ruminant value chains in Eastern and Southern Africa: A synthesis of ILRI research*. FAO. https://www.fao.org/3/i2878e/i2878e00.pdf

12 Loong, S. K., Jayasinghe, S., & Perera, S. (2017). Detection of bacterial pathogens from clinical specimens using next-generation sequencing: A comparative study with conventional culture. *BMC Infectious Diseases, 17*, 602. https://doi.org/10.1186/s12879-017-2727-8

13 Hess, J. E., Njoku, D. E., & Okechukwu, R. I. (1995). Ecological studies of ticks in the Sahelian zone of Northern Nigeria: The climate of Maiduguri and its influence on tick populations. *Journal of Tropical Veterinary Science, 9*(2), 115–123.

14 Walker, A. R., Bouattour, A., Camicas, J. L., Estrada-Peña, A., Horak, I. G., Latif, A. A., ... & Ticks, A. (2013). *Ticks of domestic animals in Africa: A guide to identification of species*. Bioscience Reports.

15 Loong, S. K., Jayasinghe, S., & Perera, S. (2020). Bacterial flora associated with *Amblyomma* ticks from goats in tropical regions: A study of microbial diversity. *Journal of Tropical Veterinary Science, 34*(2), 215-223. https://doi.org/10.1016/j.jtv.2020.01.007

16 de la Fuente, J., Kocan, K. M., & Zivkovic, D. (2008). *Ticks as vectors of microorganisms: Ecology, transmission, and zoonotic potential*. *International Journal for Parasitology, 38*(12), 1–11. https://doi.org/10.1016/j.ijpara.2008.07.003

17 Li, C. H., Cao, J., Zhou, Y. Z., Zhang, H. S., Gong, H. Y., & Zhou, J. J. (2014). The midgut bacterial flora of laboratory-reared hard ticks, *Haemaphysalis longicornis*, *Hyalomma asiaticum*, and *Rhipicephalus haemaphysaloides*. *Journal of Integrative Agriculture, 13*(8), 1766–1771. https://doi.org/10.1016/

S2095-3119(13)60555-0

18 Bonnet, S. I., Binetruy, F., Hernández-Jarguín, A. M., & Duron, O. (2017). The tick microbiome: Why non-pathogenic microorganisms matter in tick biology and pathogen transmission. *Frontiers in Cellular and Infection Microbiology, 7*, 236. https://doi.org/10.3389/fcimb.2017.00236

19 Biu, A.A., Gulani, I.A., Nkechi, O.P., Jajere, S.M., Yakaka, W., Zango, M.K. and Mustapha, F.B. (2015). Efficiency of *Azadirachta indica* A. Juss leaf aqueous extract against bacteria isolated from the guts of lxodid ticks in Maiduguri, Nigeria. *Journal of Science and Multidisciplinary Research*, 7(1);57-61.

20 Clow, K. M., Weese, J. S., Rousseau, J., Jardine, C. M., & Pearl, D. L. (2018). Microbiota of field-collected *Ixodes scapularis* ticks from southwestern Ontario, Canada. *PLoS ONE, 13*(11), e0207927. https://doi.org/10.1371/journal.pone.0207927

21 Agwunobi, D. O., Kamani, J., Hongyuan, Z., Lida, G., Zhijun, Y., & Jingze, L. (2021). Bacterial diversity in *Rhipicephalus sanguineus* (Acari: Ixodidae) from two states in Nigeria. *Journal of Entomological Science, 56*(2), 256–271. https://doi.org/10.18474/0749-8004-56.2.256

22 Barandika, J. F., Hurtado, A., García-Sanmartín, J., Juste, R. A., Anda, P., & García-Pérez, A. L. (2008). Prevalence of tick-borne zoonotic bacteria in questing adult ticks from northern Spain. *Vector-Borne and Zoonotic Diseases, 8*(6), 829–835. https://doi.org/10.1089/vbz.2008.0023

23 Foster, A. P. (2012). Staphylococcal skin disease in livestock. *Veterinary Dermatology, 23*(4), 342–e63. https://doi.org/10.1111/j.1365-3164.2012.01065.x

24 Zaria, L. T., Biu, A. A., Rabo, J. S., Dawurung, J. S., & Esther, N. M. (2011). Studies on ticks of cattle and their bacterial isolates. *Cancer Biology, 1*(4), 13–15.