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Comparism of Microscopy and Molecular Diagnosis of *Ehrlichia ruminantium* in Cattles in Makurdi Benue Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Makurdi, is the capital city of Benue State, the food basket of the Nigeria, the city challenged with *Ehrlichia ruminantium* of cattle that are responsible for severe losses caused either by negative impact of ticks blood loss or blood related infections, damage to hides and others.
Aim: This study was designed to identify and Characterize *Ehrlichia ruminantium* of Cattle within Makurdi Nigeria using microscopy and molecular techniques (PCR).
Methodology: Blood sample were collected from a total of 432 cattle of both sexes and analyzed microscopically using thin blood film and DNA examination was done using polymerase chain reaction (PCR). Tick-borne pathogens were identified and characterized by PCR amplification using species specific primer of 16s rRNA for *Ehrlichia*. Data obtained were analyzed using chi square, t-test and P values at less than 0.05 were considered significantly different.
Results: The result of comparison of prevalence of infection of haeomoparasite using microscopy and PCR revealed that, microscopy was not able to detect *Ehrlichia* while PCR yielded at percentage of 15%, *Ehrlichia* prevalence. The percentage of prevalence of haemoparasite was highly detected by PCR than microscopy and this was statistically significant ($P < 0.05$).
Conclusion: *Ehrlichia ruminantium* in cattle have been seen to be prevalent in Makurdi metropolis and molecular method such as PCR can effectively diagnose the infection.

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1. BACKGROUND

Ehrlichia ruminantium (also known as heart water) is a tick-borne rickettsial disease of domestic ruminants such as sheep, goats, cattle and wild ruminants such as buffalo, giraffe, and antelope as well as some wild rodents. It is an intracellular Gram-negative coccal bacterium (also referred to as *Rickettsia ruminantium*). The disease is spread by bont ticks, which are members of the genus *Amblyomma*. The disease has the biggest economic impact on cattle production in affected areas. The disease's name is derived from the fact that fluid can collect around the heart or in the lungs of infected animals. [1] Ticks attach to their hosts and facilitate transmission of infectious agents to different geographical regions via traveling pets, migration of animals or other means of transportation [2]. Often time some of the heart water diseases appear very difficult to diagnose, mainly by microscopy. Makurdi, Benue Nigeria has many livestock farmers who are faced with the challenge of heart water diseases which are difficult to diagnose using microscope, hence the need to use PCR diagnostic method.

1.1 Sample Analytical Procedure

The blood samples were collected using standard procedure and were analyzed using microscopic method (Thin blood film method) and polymerase chain reaction (PCR).

1.2 DNA extraction

DNA extraction was performed using zymo quick - DNA^{TM} miniprep plus kit based on the manufacturer's instructions protocol for DNA extraction. Dilution was as followed. 3 mm circler portion of each Whatman fitter paper 3 were punched using a Harris micro punch and placed in a microcentrifuge tube containing a solution of 95 μl water, 95 μl of solid tissue lysis butter and 10 μl proteinase K were thoroughly mixed and incubate at 56 ^{o}C for 3 hours until the whatman fitter papers becomes insoluble debris and pellet by centrifugation at12,500 Xg for 1 minute. The aqueous supermatant was transfer to a clean tube and 400 μl of Genonic Binding Butter was added to 200 μl supernatant and mix thoroughly, the mixture was transfer to a zymo-spin^{TM} 11C-XL Column in a collection tube, centrifuge at 13,000 xg for 1 minute and discard the collection tube with the flow through, 400 μl DNA pre-wash Butter was added to the column in a new collection tube and centrifuge for 1 minute. The collection tube was empty and 700 μl g DNA wash Butter was added and centrifuge for 1 minute and collection tube empty 200 μl of g - DNA wash Butter was added and centrifuge for 1 minute, the flow through was discard with the collection. The column was transfered to a new collection, all DNA eluted with 65 μl added to the spin column and incubated for 5 minutes; at 13,000 xg. The eluted DNA was stored at **<**-20^{o}C before amplification with PCR.

1.3 Identification of *Ehrlichia ruminantium* Pathogens using PCR Amplification

The pathogens were identified and characterized by PCR amplification using species specific primers for different pathogens. The forward and reverse primers which are species specific primers for PCR amplification of 18s rRNA1. The amplification was performed in an automated thermo-cycler with an initial denaturation step at 95 ^{o}C for 3 min. followed by 30 cycles at 94 ^{o}C for 30s, 59 ^{o}C for 1 min and 72 ^{o}C for 1 min, annealing 45s at 55 ^{o}C with a final extension step of 72 ^{o}C for 5 minutes. The resulting amplified products was electrophoresed on a 2 % agarose gel at 95 V for 45 minutes, stained with ethidium bromide at 100 bp molecular weight masses was included to identify the bed weight. The amplified product was visualizes under a UV transmilluminator and photographed with a digital camera.

1.4 Statistics

Data obtained was analyzed using Chi-square test, to determine whether there is a significant different between the expected frequencies and the observed frequencies in one or more categories and to examine differences within categorical variables. PCR was compared with the diagnostic performance of microscopy by calculating sensitivity, specificity, positive predictive value and negative predictive value.

Sensitivity is calculated as: (Number of true positive / Number of true positives + Number of false negatives)

Specificity is calculated as: (Number of true negative / Number of true negative + Number of false positive)

Positive predictive value = (Number of true positive / Number of true positive + Number of false positive)

Negative predictive value = (Number of true negative / Number of false negative + Number of true nagetive)

2. RESULTS

The result of diagnosis of *Ehrlichia ruminatium* with microscopy using PCR is presented in Table 2. The sensitivity of PCR to *Ehrlichia ruminatium* yielded 0.0 % estimated value and 0 % lower limit on 95 % confidence interval but 37.1 % upper limit.

The specificity produced 1 % estimated and at lower limit PCR yielded 1 % specificity and 91.3 % at upper limit. However, there was no case of PPV at estimated, lower and upper limit on 95 % confidence interval. There was high record of NPV of 85 % estimated and 72.9 % lower limit and 92.5 % upper limit.

Plate 1 shows the representation of agarose-gel electrophoresis result showing primary reaction of *Ehrlichia ruminantium* positive samples amplified at 430 base pairs as shown by the white thick band between 400 base pair and 500 bed pairs. The DNA ladder reads at lane 1, 2, indicate Negative sample of *Ehrlichia ruminantium* and lane 3, 4, 5, 6, 7, 8, 9, 10 11 indicate positive cattle sample. Lane 12 indicates control.

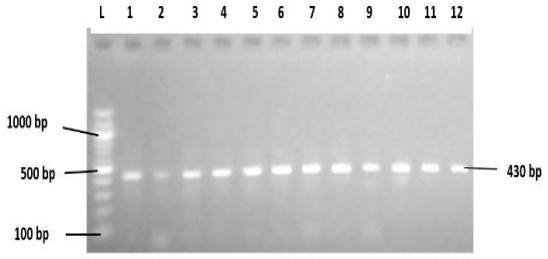

Plate 1. Representation of agarose-gel electrophoresis result showing Primary reaction of Ehlrichia species 430 bp fragment

Plate 2 is representation of agarose-gel electrophoresis result showing secondary reaction of *Ehrlichia ruminatium* positive sample amplified at 201 bed pairs as shown by the thick white band between 200 bed pair and 300 bed pair. The DNA ladder reads at lane 1, 2, 3, 4, 5, 6 which indicate positive samples of *Ehrlichia ruminatium* in cattle and lane 8 indicate negative sample. Lane 9 indicates control.

Table 1. List of universal and species specific primers used for PCR amplification of 16s rRNA (Ehrlichia)

Parasite Speciese	Primer Sequence	Amplian size (bp)
Primer Ehrlicha, Universal AnE – F AnE – R	*F:5[1]_ GGTTTAATTCGATGCA ACGCGA – 3'R;5 – CGTAT TCACCGTGGC ATG – 3[1]*	*430*
Species specific E. ruminantium ER – R	*R:5[1]_ GAGTGCCCAGCATTA CCTGT – 3*	*201*

Table 2. Diagnostic parameters of *Ehrlichia ruminantium* with microscopy using PCR as reference test

Ehrlichia ruminantium	Estimated Value %	95% confidence Lower limit %	Interval Upper limit %
Sensitivity	0	0	37.1
Specificity	1	1	91.3
PPV	*Nil*	Nil	Nil
NPV	*85*	72.9	92.5

3. DISCUSSION

PCR method was able to detected *Ehrlichia ruminantum* which was not detected microscopically. This is an indication that false positive sample and false negative sample have been recorded using microscopy as Vahid (2014) and Abanda et al*.,* (2019) stated that microscopy is most used for the identification of piroplasma and it accompanied with some technical problems which leads to false morphological diagnosis. Thus, this method is not sensitive enough or sufficiently specific to detect chronic infection of cattle with heart water diseases.

The study revealed the presence of *E. ruminantium*. However, in Nigeria particularly Makurdi, identification and characterization of tick-borne haemoparasitic diseases using molecular method (PCR) are scarce however, using microscopy, many researchers have reported the occurrence of *E. ruminantium* in cattle and other ruminant. Stuen et al., [4], Zhou et al*.,* [5] reported that heartwater disease can result in public health and economic consequences to cattle rearers.

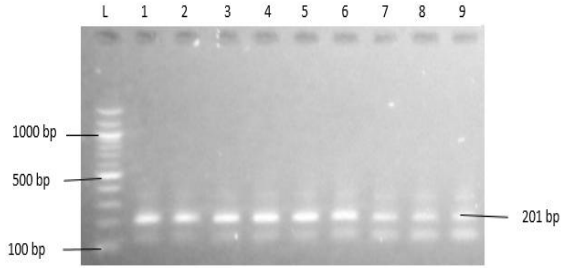

Plate 2. Representation of agarose-gel electrophoresis result showing Secondary reaction of Ehrlichia ruminatium lower panel = 201 bp fragment

This study used the same species specific primers used for PCR amplificatuion of 16s rRNA genes for *Ehrlichia ruminantium* as used by Jalali et al.[6] for detecting pathogens by PCR.

Thus, the species specific primers have been used for detection and identification of both plant and animals pathogens by PCR hence this indicate it could be widely used to develop molecular techniques for defection of other pathogens of veterinary and medical importance [7]. Undoubtedly from the literature, these appear to add to the baseline data in which primers are designed using species specific gene for the detection of tick-borne haemoparasites.

The 16s rRNA gene sequence primer for Ehrlichia from this study shared high identity (100%) with samples collected from cattle. 16s rRNA gene fragment for Ehrlichia was bonds at approximately 201 bp for Ehrlichia. This result is in line with the study conducted by Aaron et al. [8] who also reported detection of pathogenic *Theileria, Anaplasma* and *Ehrlichia* species on their study on Molecular detection and genetic characterization of pathogenic *Theileria, Anaplasma* and *Ehrlichia* species among apparently healthy sheep in central and Western Kenya. Many reports have documented these gene markers to be used for understanding and of the molecular epidemiology of bovine *Ehrlichia* species and other diseases associated with tick-borne haemoparasite [9,10]. The result reveals the high sensitivity and specificity of PCR in identification and characterization of tick-borne haemoparasite. Therefore, PCR is reported to be characterized by high specificity. Specificity and sensitivity thus being able to identify *E. ruminantium* from other species of tick-borne haemoparasites which was not detected by microscopy.

In comparison of molecular tools to microscopic analyses of blood smears, blood microscopy is used for rapid diagnosis and informative purposes on animals health statutes, and again, identification by microscopy is prone to errors in species identification, as pathogen may look very similar among and between genera leading to misidentification, or may be missed depending on the animals patency or developmental status.

In Comparison between Polymerase chain reaction (PCR) and Microscopy, PCR yielded higher sensitivity and specificity in identification of tick-borne haemoparasite than microscopic diagnostic methods. This could be because PCR methods are gene specific and the primers are developed for particular species. It is an indication that PCR probability has a higher ability in detecting true negative samples.

The relatively higher detection rate with 16S rRNA gene as compared to microscopy suggest that the marker is highly sensitive and specific for *Ehrlichia* detection.

PCR diagnostic method was able to detect *E. ruminantium* while microscopy was not able to identify the organism due to low sensitivity. Sex, age, breed and location may affect the infection of bovine *Ehrlichia.*

4. CONCLUSION

Conclusively, PCR diagnostic method is more sensitive than microscopy in the detection of *E. ruminantium.* Therefore, microscopy alone is not okay to be used in diagnosis of *E. ruminatium* as it can give a false negative result.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Salih DA, El Hussein AM, Singla LD. Diagnostic approaches for tick-borne haemoparasitic diseases in livestock. Journal of Veterinary medicine and Animal Health. 2015;7 (2):45-56.
2. Stephanie LR, Ricky L, Charles SA, Elizabeth W. Do tick attachment times vary between different tick-pathogen systems. Journal Environments. 2017;4(3):7-13.
3. Zerihun H, Krücken J, Baumann M, Ahmed JS, Clausen PH, Nijhof AM. Molecular detection of tick-borne pathogens in cattle from Southwestern Ethiopia. PLoS ONE. 2017;12(11): 43-49.
4. Stuen S. Tick-borne infections in small ruminants in northern Europe. Ticks and Tick-borne Diseases. 2013;110(3):142-144.
5. Zhou M, Cao S, Sevinc F, Ceylan MC, Franck P, Moumouni A, Jirapattharasate C, Liu M, Wang G, Iguchi A, Vudriko P, Suzuki H, Xuan X. Molecular detection and genetic identification of *Babesia bigemina*, *Theileria annulata*, *Theileria orientalis* and *Anaplasma marginale* in Turkey. Ticks and Tick-borne Diseases. 2016;7(1):126-134.
6. Jalali SM, Khaki Z, Kazemi B, Bandehpour M, Rahbari M, RaziJalali M, Yasini SP. Molecular Detection and Identification of *Anaplasma* species in sheep from Ahavz, Iran. Iranian Journal of Veterinary Research. 2013;14(142):50-56.
7. Happi AN, Osifade O, Oluniyi PE, Ogunro BN. Comparison of Light Microscopy and Polymerase Chain Reaction for the Detection of Haemoparasites in Cattle in Nigeria. Acta Parasitologica. 2020;65:44–56.
8. Aaron ER, Gabriel OA, Paul F, Adjou M, Seung HL, Charoonluk J, Mingming L., Yang, G., Huanping G, Weiqing Z, Arternis E, Eloiza M, et al. Molecular detection and genetic characterization of pathogenic Theileria, Anamplasa and Ehrlicha species among apparently healthy sheep in central and Western Kenya. Ondertepoort Journal of Veterinary Research. 2019;86(1):1839–1847.
9. Ybañez AP, Ybañez RHD, Yokoyama N, Inokuma H. Multiple infections of Anaplasma platys variants in Philippine dogs. Vet World. 2016;9(12):1456–1460.
10. Wei F, Song M, Liu H, Wang B, Wang S, Wang Z, Ma H, Li Z, Zeng Z, Qian J, Liu Q. Molecular Detection and Characterization of Zoonotic and Veterinary Pathogens in Ticks from Northeastern China. Frontine Mircobiology. 2016;7:1913-1919.

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