



## INTERNATIONAL JOURNAL OF SCIENCE FOR GLOBAL SUSTAINABILITY

### Molecular Characterization of Bovine *Fasciola* Species from Kebbi State, Nigeria

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Received on: April, 2023

Revised and Accepted on: May, 2023

Published on: July, 2023

#### ABSTRACT

Current phenotypic techniques fail to reflect the full extent of the diversity of *Fasciola* species. Methods of diagnosis and differentiation of *Fasciola* species have been developed, but the understanding of the distribution and speciation of this parasite is location dependent. Therefore, it is important to characterize the *Fasciola* species from different geographical locations. This research was conducted to genetically characterize bovine *Fasciola* species from cattle brought for slaughter at Birnin Kebbi Modern Abattoir. Faecal samples were collected from the cattle before slaughter and were microscopically examined using two methods of Parasitological examinations (Flukefinder® Kit and formalin-ether sedimentation). Positive faecal samples were subjected to molecular detection method of PCR and sequencing of the products. The obtained sequence was subjected to BLAST and phylogenetic analysis was conducted to determine the *Fasciola* species prevalent in the area. The PCR results have detected *Fasciola* spp while the phylogenetic analysis have confirmed the species of liver fluke to be *Fasciola gigantica*. This have intensified the understanding of the existence of the parasite in the study area and have also provided the genetic diversity of *F. gigantica*. There is need for further research to compare *Fasciola* isolates from other livestock in order to determine the exact species infecting animals in the study area.

**Keywords:** *Fasciola* species, Molecular detection, Characterization, Modern Abattoir, Kebbi State, Slaughtered Cattle.

#### 1.0 INTRODUCTION

Ruminants are one of the most essential resources possessed by many families in the tropical regions of the world. These animals offer valuable animal protein for human consumption and livelihood for several families in form of livestock trade (Masoud *et al.*, 2020). *Fasciola* infection in cattle constitutes a major hazard to the livestock industry, especially in areas where favorable conditions of humidity and temperature are predominant (Fesseha and Asefa, 2022). Even though, it is impossible to measure the exact economic losses caused by bovine fasciolosis, it is a fact that it constitutes one of the primary causes of poor performance in many areas (Elelu and Eisler, 2018).

Domestic animals such as cattle have an important part in human nutrition and socio-economic development. Protein, calories, calcium, and micronutrients are abundant in milk and meat provides 28 % of protein and 13% of

calories globally (Howell *et al.*, 2020). Cattle meat products (Beef) are not only a reliable supply of protein, but also a reliable source of income and employment for butchers in developing countries such as Nigeria (Akinaw, 2021). These animals not only provide traction power and manure for crop production, but also serve as a source of capital strength for future investment (Howell, 2023). Keeping up with these new demands is becoming a difficult task that will rely heavily on improving the efficiency of components such as the production system, pasture and disease control strategies (Frigerio *et al.*, 2020).

Detection of *Fasciola* egg in the faeces of infected animal using conventional parasitological methods cannot distinguish *Fasciola* spp from other trematodes when there is mixed infection. This occurs due to morphological similarities between egg of various trematode genera and species, making

accurate species-specific diagnosis difficult (Addy *et al.*, 2020). In addition, this problem has made the investigation on *Fasciola* and other trematode species difficult especially in some of the tropical regions where they co-exist. Similarly, literature is limited on the *Fasciola* species diversities in Nigeria especially in the study area (Kebbi State) where indigenous and exotic mixed cattle production is a common practice. The exotic cattle breed in the area could have introduced other *Fasciola* species to the local population. This study is aimed to characterize bovine *Fasciola* species prevalent in the study area.

## 2.0 MATERIALS AND METHODS

### 2.1 Study Area

Kebbi State is one of the major livestock producer in Nigeria. An estimation of 1.8 million cattle was previously documented (MAHF&H, 2018). Cattle serves a purpose of meat, milk and dung (manure) for crop farming. The State is located between the latitude of 10° and 30° North and longitude 3° and 6° East. It shares international borders with the Republic of Benin and the Republic of Niger to the West and internal borders with Sokoto and Zamfara State on the North, and Niger State to the South. The State occupies approximately 36, 800 square kilometers. Over 75% of the human populations reside in rural areas with farming as their major occupation (MAHF&H, 2018).

### 2.2 Sample Collection and Transportation

Rectal faecal samples were freshly collected from each sampled cattle ante-mortem using polythene bags. The samples (20 grams of faeces) were then transported on ice in a plastic container to the Laboratory of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto for processing and identification.

### 2.3 Parasitological examinations

Collected faecal samples were microscopically examined using two different concentration methods (Formalin-ether Sedimentation Technique and Flukefinder® Kit.) for fluke eggs detection using their morphology.

### 2.4 Molecular Characterisation of *Fasciola* Species

DNA was extracted from all faecal samples positive for *Fasciola* eggs in both methods.

#### 2.4.1 Genomic DNA Extraction from Faeces of *Fasciola* Infected Cattle

QIAamp® DNA stool MiniKit (QIAGEN, Hilden, Germany) was used for genomic DNA extraction from the positive faecal samples as previously documented (Mahmuda *et al.*, 2017).

#### 2.4.2 Detection of 28S rDNA Gene of *Fasciola* species

*Fasciola* spp specific primer set, was designed to amplify partial ribosomal DNA (28S) of the genome. The Forward (5'-ACC GGT GCT GAG AAG ACG-3') and Reverse (5'-CGA CGT ACG TGC AGT CCA-3') based on rDNA sequences related to *Fasciola* spp were used to amplify a 463 bp fragment. PCR amplification was achieved using the following reaction mix: 12.5 µl of 1x Top Taq Master Mix (Qiagen, USA), 0.5 µl of each primer pair (20 mM), 5µl of template DNA and 6.5 µl of nuclease-free water to make up to final volume of 25 µl. In the negative control tube, 5 µl of nuclease free water was used as template. The tubes were transferred into applied Bio-system 9700 thermal cycler programmed with the following cycling conditions: initial denaturation at 94°C for 1 minute and 30 seconds, followed by 40 cycles of denaturation at 94°C for 1 minute and 30 seconds, 53°C for 1 minute and 30 seconds (annealing) and 68°C for 2 minutes (extension), followed by extension (final) of 68°C for 5 minutes (Imani-Baran *et al.*, 2017).

#### 2.4.3 Gel Electrophoresis of PCR Products

The 1.5% Agarose gel was prepared. Approximately 10µl of each PCR product, Negative control and 5µl of 100-bp DNA ladder (DSB, China), were carefully loaded in allocated wells. Electrophoresis was carried out for 1hr at 70 volts. The gel slab was carefully transferred to a gel documentation device and visualized under UV trans-illuminator (Bio-Rad, Germany), and photographed, stored and sample wells were observed for the expected amplicon size corresponding to the 463-bp band of the DNA ladder.

#### 2.4.4 Gene Sequencing and Alignment

PCR products from representative samples were sealed and packaged on ice pack and sent for purification and sequencing (INQABA, South

Africa). The chromatograms of the sequences received were accessed using Molecular Evolutionary Genetics Analysis 11 (MEGA 11) software. The FASTA (Fast-All) format of each nucleotide sequence was obtained, uploaded and aligned with those from the GenBank database to obtain the percentage of homology using Basic Local Alignment Software Tool (BLAST) ([www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST)).

### 3.0 RESULTS

#### 3.1 Parasitological Prevalence of Fluke Infection

A total of sixteen (16) samples out of seventy three (73) samples were found to be positive for fluke eggs in both parasitological methods employed which were considered for molecular detection and characterization.

#### 3.2 Molecular Characteristics of Bovine Isolates of *Fasciola* Species

Following PCR amplification of the extracted DNA from sixteen (16) faecal samples, a 463 bp band was obtained via electrophoresis of the PCR products. There was amplification in the eleven (11) samples processed, given a detection rate of 68.8% but no evidence of amplification in five (5)

of the samples. Absence of band in the negative control (NC) is an indication that there was no contamination in the reaction (Figure 1).

#### 3.3 Similarity (BLAST) Tests Results of two Bovine Isolates of *Fasciola* Species

A 463 bp fragment from two (2) representative specimens were sequenced. According to similarity test results for percentage homology of the local sequences in comparison with the available data of reference *Fasciola* spp registered in GenBank database, where the sequence under accession number MT644663.1 had shown a 100% homology with the local sequence GB5 GB while KY613943.1 showed a 99% homology with the local sequences (4GBR) in the present study (Figure 2).

#### 3.4 Phylogenetic Analysis Results of two Bovine Isolates of *Fasciola* Species

The phylogenetic tree showed that the query sequence (GB5 GB present study) is observed to be clustered with the *F. gigantica* reported in Nigeria (MT644664.1), Iran (MF969004.1), Turkey (KY613943.1) and Vietnam (MN970009.1) (Figure 2).

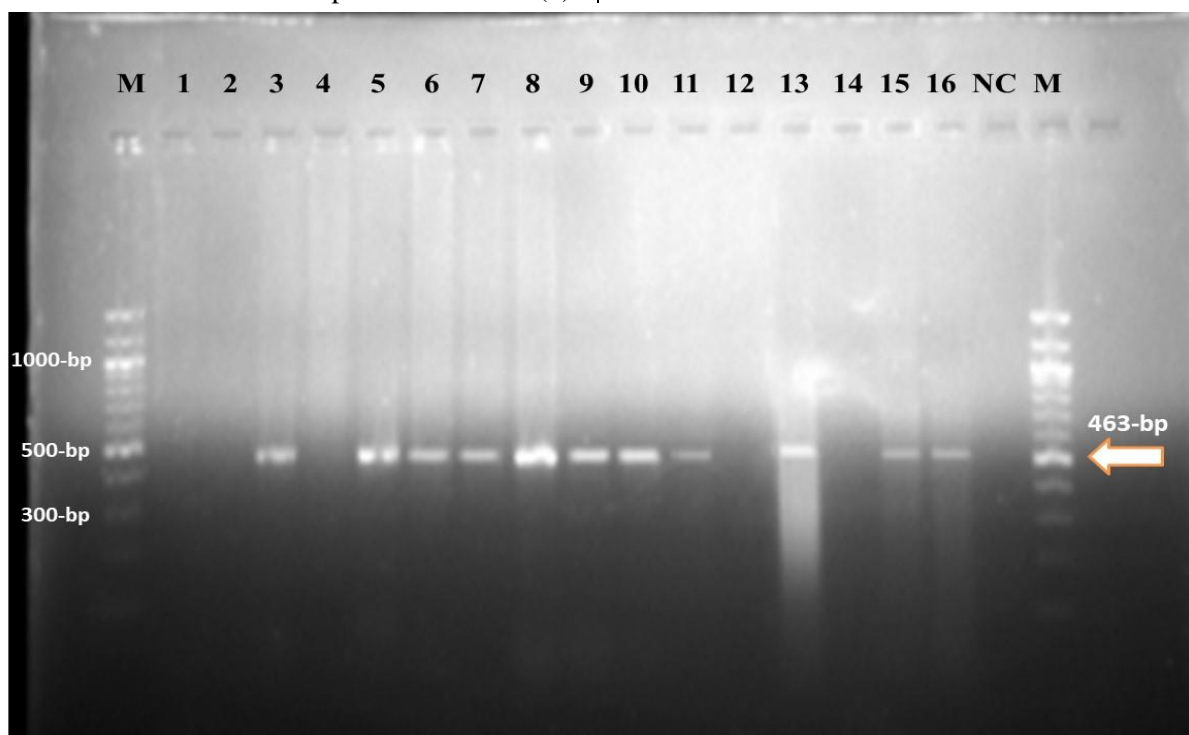
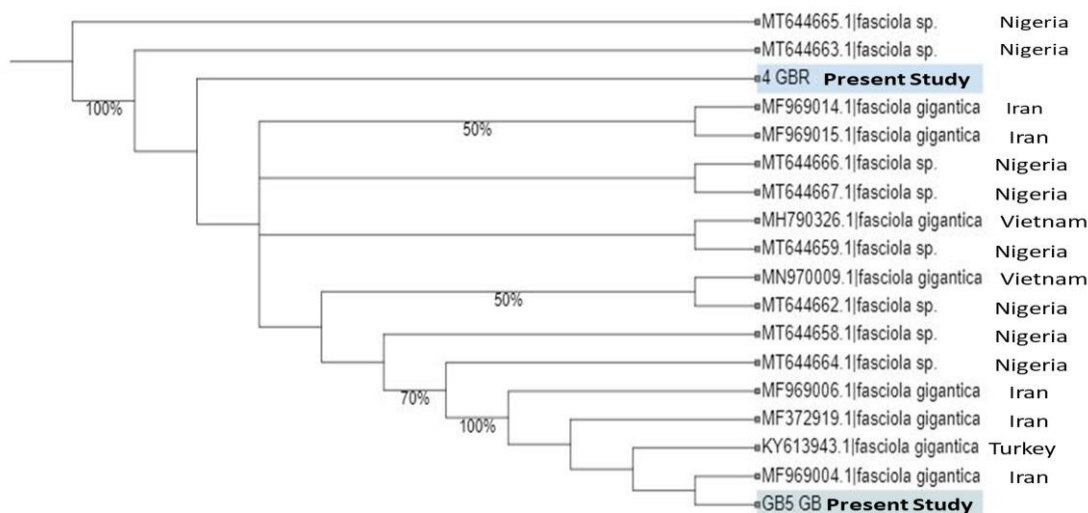


Figure 1: Agarose Gel Image Showing Amplification of 463-bp fragment of 28S rDNA of *Fasciola gigantica*. M=DNA Ladder; 1-16= Samples; NC= negative control



**Figure 2: Phylogenetic relationship of 28S rDNA gene sequences of two isolates (present study) of *Fasciola gigantica* from slaughtered cattle at the Birnin Kebbi Modern Abattoir, using the Maximum likelihood, Method.**

#### 4.0 DISCUSSION

Several researchers suggested that *Fasciola gigantica* and *Fasciola hepatica* are the two predominant *Fasciola* species associated with livestock infection in Nigeria and indeed the majority of the West African region (Addy *et al.*, 2020). This study further supports the long-established fact. However, there are contradictory reports regarding the species that are commonly isolated from cattle in Nigeria (Elelu and Eisler, 2018). While the majority of reports indicate that *F. gigantica* is more prevalent among cattle, some reports differ by identifying *F. hepatica* as the dominant species in their respective areas (Castilla Gómez de Agüero *et al.*, 2020).

Several studies have documented molecular prevalence of *Fasciola* species in cattle and in ruminants in Nigeria (Castilla Gómez de Agüero *et al.*, 2020; Salihu *et al.*, 2022) and different parts of the world (Hamoo *et al.*, 2019; Giovanoli *et al.*, 2020; Rokni *et al.*, 2020). Of the Sixteen (16) selected representative samples for PCR amplification, five (5) were negative which were evident by absence of band in the reaction. This negative samples could be eggs from other trematodes as have previously been documented (Alajmi, 2019).

The phylogenetic results confirm that *Fasciola* spp in cattle in the current study area (Kebbi State) are similar to those identified in Sokoto State, Nigeria (Salihu *et al.*, 2022) and are *Fasciola gigantica*. The same authors recorded similarity with other sequences deposited from

Niger Republic, Burkina Faso, and Zambia and conclusion was drawn about the possibility of cross-country transmission and local extension of the parasite which may be as a result of the traditional nomadic migration (Salihu *et al.*, 2022). Similarly, the phylogenetic analysis results also confirmed similarities between the sequence in this study with those found in other countries, including Iran, Vietnam, and Turkey.

With the type of informal livestock trade in the region of West Africa, the animal movement patterns remain unrecorded (Salihu *et al.*, 2022). Unfortunately, these migrations are characterized by widespread incidence of economically devastating infectious diseases, including fasciolosis (Rokni *et al.*, 2020). The findings in this study conform with results of a similar study in Iran and also other African countries where circulating *Fasciola* species were attributed to transboundary livestock migration (Ichikawa-Seki *et al.*, 2017; Saadtania *et al.*, 2022). Indeed, alignment results for the obtained sequences from the present study is in agreement with those that reported *F. gigantica* as has also been previously documented (Castilla Gómez de Agüero *et al.*, 2020).

#### 5.0 CONCLUSION

This study was able to demonstrate and conclude that the liver fluke isolated from slaughtered cattle in Birnin Kebbi Modern Abattoir is *Fasciola gigantica*. The study is the first to explore the molecular characteristics of *Fasciola* in the study area. From the outcome of this investigations, it can be concluded that this region



of the gene (28S rDNA) is a good target for molecular detection and characterization of *Fasciola* species.

## 6.0 RECOMMENDATIONS

Further molecular identification of other fluke species will provide more information about the prevalence of digenic trematodes infecting cattle in the study area. There is need to also determine the geographical origin of the slaughtered cattle at the abattoir to enhance the interpretation of the phylogenetic analysis.

## 7.0 ACKNOWLEDGEMENTS

The efforts of all authors and laboratory technicians in the collection, transportation, processing of the samples, analysis of results and manuscript drafting are hereby acknowledged.

## REFERENCES

- Addy, F., Gyan, K., Arhin, E. and Wassermann, M. (2020). Prevalence of Bovine Fasciolosis from the Bolgatanga Abattoir, Ghana. *Scientific African*, **8**, p.e 00469. <https://doi.org/10.1016/J.SCIAF.2020.E00469>.
- Akinaw, W. (2021). A Review on cattle Fasciolosis. *Journal of Veterinary Medicine and Surgery*, **5** (4):6740. P1-6.
- Alajmi, R.A. (2019). Molecular Characterization of *Fasciola* Flukes Using Mitochondrial 28S rRNA Gene in Naimi Saudi Sheep. *Saudi Journal of Biological Sciences*, **26** 112-117. doi: 10.1016/j.sjbs.2017.06.010
- Castilla Gómez de Agüero, V., Luka, J., Gandasegui, J., Valderas-García, E., Ajanusi, O.J., Chiezey, N.P. and Martínez-Valladares, M. (2020). *Fasciola hepatica* and *Fasciola gigantica* Coexistence in Domestic Ruminants in Nigeria: Application of a PCR-Based Tool. *Tropical Animal Health and Production*, **52** (6):3893-3897. DOI: 10.1007/s11250-020-02333-3
- Elelu, N. and Eisler, M.C. (2018). A review of bovine fasciolosis and other trematode infections in Nigeria. *Journal of Helminthology*, **92** (2): pp.128-141. doi: 10.1017/S0022149X17000402.
- Fesseha, H. and Asefa, I. (2022). Co-infection of fasciolosis and hydatidosis and their financial loss in cattle slaughtered at Wolaita Sodo municipal abattoir, southern Ethiopia. *Animal Diseases*, **27**. <https://doi.org/10.1186/s44149-022-00059-3>.
- Frigerio, S., Da Costa, V. & Costa, M. (2020). Eosinophils control liver damage by modulating immune responses against *Fasciola hepatica*. *Frontiers in Immunology*, **11**:579801.
- Giovanoli, E. J, Schmidt, R.S., Boltryk, S.D., Voss, T.S., Batil, A.A., Ngandolo, B.N., Greter, H., Utzinger, J., Zinsstag, J., Balmer, O. (2020). Molecular Confirmation of a *Fasciola gigantica* × *Fasciola hepatica* Hybrid in a Chadian Bovine. *Journal of Parasitology*, **106** (2):316-322. doi: 10.1645/19-66. PMID: 32330281.
- Hamoo, RN, Al-Rubaye, FSI, Mustafa, NG (2019). Molecular characterization and phylogenetic analysis of *Fasciola gigantica* in Iraqi sheep using ITS1. *Advances in Animal and Veterinary Science*, **7**(4):256-260.
- Howell, A.K., Malalana, F., Beesley, N.J. Hodgkinson, J.E. Rhodes, H., Sekiya, M., Archer, D., Clough, H.E., Gilmore, P. & Williams, D.J.L. (2020). *Fasciola hepatica* in UK horses. *Equine Veterinary Journal*, **52**(2):194-199. DOI: 10.1111/evj.13149.
- Howell, A. (2023). Snail-borne diseases in bovids at high and low altitude in Eastern Uganda: Integrated parasitological and malacological mapping.
- Ichikawa-Seki, M., Tokashiki, M., Opara, M.N., Iroh, G., Hayashi, K., Kumar, U.M. and Itagaki, T. (2017). Molecular characterization and phylogenetic analysis of *Fasciola gigantica* from Nigeria. *Parasitology International*, **66** (1): 893-897.
- Imani-Baran, A., Habib, C. S. and Farzad, K. (2017). Molecular Determination of *Fasciola* spp. Isolate from Domestic Ruminants Fecal Samples in the North-west of Iran. *Iranian Journal of Veterinary Parasitology*, **12**: 243-250.
- Mahmuda, A., Al-Zihiry, K.J.K., Roslaini, A.M., Rukman, A.H, Abdulhaleem, N., Bande, F., Mohammed, A.A., Alayande, M.O., Abdullah, W.O. and Zasmy, U. (2017). Histopathological confirmation of disseminated larvae (iL3) of *Strongyloides ratti* in an immunosuppressed Wistar rat. *Tropical Biomedicine*, **34** (1): 212-223.
- Masoud, S., Mahdi, F., Saeed, H., Teshnizi, R. Z., Enameh, H. Z., Hezarjaribi, S. A., Roghiyeh, F. & Hamed, K. (2020). Status of fasciolosis among domestic ruminants in Iran based on abattoir data: a systematic review and

- meta-analysis. *Annals of Parasitology*, 66(1), 77–86. doi: 10.17420/ap6601.240.
- Ministry of Animal Health, Fisheries and Husbandry (2018). Kebbi State Government. To add value to human existence through livestock production and boost the economy of Kebbi State. @kebbistate.gov.ng.
- Rokni, M.B., Bashiri, H., Raeghi, S., Teimouri, A., Shojaeimotlagh, V., Shiee, M.R., Bozorgomid, A. (2020). Molecular phylogenetic and genetic variability of *Fasciola gigantica* in Kermanshah province, western Iran with an overview to understand haplotypes distribution in Asia and Africa. *Veterinary Research Forum*, 11 (3): 265 – 271.
- Saadatnia, A., Solhjoo, K., Mohamad Hasan Davami, M.H., Raeghi, S., and Abolghazi, A. (2022). Molecular Identification of *Fasciola* Isolated from the Liver of Meat Animals in Fars Province, Iran. *Journal of Parasitology Research*, Volume 2022, Article ID 4291230, 5 pages  
<https://doi.org/10.1155/2022/4291230>.
- Salihu, M. D., Musawa, A. I., Garba, B., Yakubu, Y., Bello, M. B., Magaji, A. A., Junaidu, A. U., Jibril, A. H., Ballah, F. M. and Achi, C. R. (2022). Molecular Characterization and Species Differentiation of *Fasciola* Parasite Isolated from Cattle Slaughtered at Sokoto Modern Abattoir, Nigeria. *Nigerian Journal of Parasitology*, 43(1), 102-108.