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Zoology

Rediscription of a species from *Capra hircus, Avitellina centripunctata* Rivolto, (1874) From Jalna distinct (M.S.), India.

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ABSTRACT The present investigation deals with rediscription of *Avitellina centripunctata* Rivolto (1874) obtained from the *Capra hircus* from Jalna District (M.S.) India. The present worm comes closer to *Avitellina centripunctata* with most of the character however, it differ from *A. centripunctata*, scolex is Quadrangular, testes are 14 to 16 in number and par uterine organ sac like. In molecular characterization of the parasites, the genomic DNA were amplified and sequenced. Based upon both morphological and molecular analysis using bioinformatics tools, the cestode is identified and confirmed to be representing *Avitellina centripunctata*, Rivolta, 1874 in mammalian host i.e. Goat.

KEYWORDS : *Avitellina, Capra hircus,* Jalna M.S.

INTRODUCTION

The genus *Avitellina* was established by Gough 1911, and its type species *A. centripunctata* (Rivolta, 1874) in *Ovis aries* in Europe. Helminths are one of the more destructive internal parasites of the vertebrate including man (Strickland, G.T., 2000). The genus *Avitellina* is an important parasitic tapeworm that has many final hosts such as cattle, sheep, goats and other domestic and wild ruminants. It is common parasite of domestic ruminants in Europe, Asia and Africa (Gary R. M., 2002). The presence of *Avitellina* species in ruminant can negatively affect their productivity. Lambs are more susceptible to infection with *Avitellina* causes diarrhea and reduced weight gain. Also it causes gastrointestinal disorders and even death in sheep and goats. So it constitutes a big problem in sheep raising countries.

Recent efforts based on morphological and molecular study of cestode tapeworm. The use of molecular data for the study of relationships among tapeworm has been largely limited to those species of medical or economic importance, although few recent studies have begun to address the systematic of a wider diversity of tapeworms. In addition to morphological characters that are often variable, difficult to homologies, molecular data have been widely used in phylogenetic studies of Cestodes generally and these Cestodes particularly using many genes and developed techniques as attempts in solving many taxonomic problem. Aim of these study was to evaluate the phylogenetic position of the Cestode *Avitellina* parasite of the Goat i.e. *Capra hircus* (L.) from Jalna district (M.S.), India on the basis of morphological and molecular data.

MATERIAL AND METHODS

Cestode parasites were collected from the intestine of *Capra hircus* at Aurangabad district (M.S.) India. These Cestodes preserved in hot 4% formalin and stained with Aceto-caramine or Harris haematoxylin, passed through various alcoholic grades, cleared in xyline, mounted in D.P.X. and drawings are made with the aid of camera lucida. All measurements are given in millimeters, otherwise mentioned. The identification is made with the help of Systema Helminthum.

Cestodes intended for molecular analysis were fixed with 95% ethyl alcohol. DNA Extraction was carried out using Genelute Mammalian Genomic DNA extraction kit (Sigma, G1N70-1KT). 25mg of tissue was minced and transferred to 1.5ml microcentrifuge tube. 180µl of Lysis solution T and 20 µl of proteinase K were added. The samples were mixed and incubated at 550C to digest the tissue completely. 20 µl of RNase A solution was added and incubated at room temperature for 2min. Then 200µl of lysis solution C was added and incubated at 700C for 10 min. The column was prepared for binding by adding 500µl of Column preparation solution to each pre-assembled GenElute Miniprep Binding Column and centrifuge at 12,000 rpmfor 1 min. 200µl of ethanol was added to the lysate and mixed by vortexing. The entire lysate was transferred into the treated binding column and centrifuge at 10,000rpm for 1 min. The binding column was then placed in fresh 2ml collection tube. 500µl of Wash solution was added to the binding column and centrifuge at 10,000 rpm for 3min. This step was repeated twice. The column was again transferred to a new tube. 200µl of elution buffer was added directly into the centre of the binding column and centrifuge at 10,000rpm for 1min. Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The DNA was stored at -200C for further use.

The DNA isolated was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). The PCR reaction mix contained 2.5µl of 10X buffer, 1µl of each primer, 2.5µl of 2.5mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1µl Template DNA and 8.5µl nuclease free water. The PCR amplification cycle consist of, a cycle of 5 min at 94°C; 35 cycles of 1min at 94°C, 1 min at 56°C, 2 min at 72°C; and additionally 1 cycle of 7 min at 72°C. The reagents used are procured from GeNei.

Gel electrophoresis was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The band size obtained for amplification of Partial 18S rRNA region is ~1000bp.

The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). 100µl of PCR-A buffer was added to the 25µl of reaction. The sample was mixed and transferred to column placed in 2ml collection tube and centrifuge at 10,000 rpm for 1min. the filtrate was discarded. 700µl of W2 buffer was added to the column and centrifuge at 10,000rpm for 2min. This step was repeated twice. The column was transferred to a new tube. 25µl of Eluent was added into the column and incubated at room temperature for 2min. Then centrifuge at 10,000rpm for 5min. It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of 18S rRNA PCR product 18s 5F- 5' (CTGGTTGATYCTGCCAGT 3') sequencing primer was used and for sequencing 28S rRNA PCR product LSU5F 5' (TAGGTCGACCCGCTGAAYTTAAGCA) sequencing primer was used.

The DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Centre for Biotechnology Information (NCBI). The BLAST results were used to find out evolutionary relationship of Worms. Altogether eleven sequences, including sample were used to generate phylogenetic tree (Figure 3). The tree was constructed by using MEGA 5 software (Saitou N. and Nei M., 1987; Felsenstein J.1985 and Tamura K. et al 2011). All this molecular work done with help of Codon Bio-science lab Panji, Goa.

DISCRIPTION

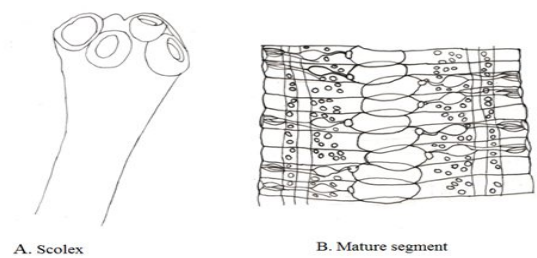
The worms were fairly large, highly muscular with numerous proglottids. The Scolex is large in size, quadrangular, distinctly marked off from the strobila, broad anteriorly and narrows posteriorly, measures 2.29 mm in length and 1.36 mm in width. The suckers are highly muscular and cup shaped arranged in a line and measures 0.03mm in length and 0.501mm in width. The neck is long and measures 1.598 mm in length and 0.867 in width.

The mature proglottids are 10 to 11 times broader than long and measures 0.532 mm in length and 5.119 mm in width. Testes are rounded, few, 14 to 16 in number evenly distributed in four columns and measures 0.051 mm in diameter. The cirrus pouch is small, elongated, cylindrical, situated at middle of the lateral margin of the segment; and measures 0.475 in length and 0.133 mm in width. Cirrus is very thin, either straight or slightly curved and measures 1.33 mm in length and 0.038 mm in width. The vas deferens is thin coiled and measures 0.131 mm in length and 0.028 in width.

The ovary is of medium size, single, compact mass, in poral half of the segment and measures 0.171 mm in length and 0.133 mm in width. Vagina is a thin tube, wider proximately and narrow distally, posterior to the cirrus pouch and open into ootype and measures 0.038 mm in length and 0.019 mm in width.

Receptaculum semini is short, reaches to ootype and measures 0.418 in length and 0.014 in width. The viteline gland is absent. Paruterine organ, in each mature segment is long, sac like in central medulla, on antiporal side of ovary, overlap on each other and measures 1.27 mm in length and 0.665 mm in width.

The genital pore is small, round, marginal, irregularly alternate and measures 0.095 mm dimeter. The longitudinal excretory canals are narrow, situated either side of the segment and measures 0.532 mm in length and 0.091 mm in width.



Figer. 1 *Avitellina centripunctata* Rivolta, 1874

Table 1: Phylogenetic neighbors of *Avitellina* sp. based on partial 18s rRNA gene sequence

Description	Max score	Total score	Query cover	E value	Ident	Accession
Avitellinacentripunctata isolate Minxian 18S ribosomal RNA gene, complete sequence	1312	1312	100%	0.0	100%	JQ609343.1
Raillietinaaustralis 18S ribosomal RNA gene, complete sequence	1051	1051	100%	0.0	94%	AF286980.1
Raillietinachiltoni 18S ribosomal RNA gene, complete sequence	1016	1016	96%	0.0	94%	AY382313.1
Lyruterinanigropunctata partial 18S rRNA gene, fragment 1	1016	1016	95%	0.0	94%	AJ555173.1
Raillietinamitchelli 18S ribosomal RNA gene, complete sequence	1014	1014	98%	0.0	93%	AY382315.1
Raillietinadromaius 18S ribosomal RNA gene, complete sequence	1011	1011	96%	0.0	93%	AY382314.1
Raillietinabeveridgei 18S ribosomal RNA gene, complete sequence	1011	1011	96%	0.0	93%	AY382312.1
Raillietinaaustralis 18S ribosomal RNA gene, complete sequence	1011	1011	96%	0.0	93%	AY382311.1
Raillietinamicracantha partial 18S rRNA gene, fragment 1	996	996	95%	0.0	93%	AJ555178.1
Raillietinatunetensis isolate A 18S ribosomal RNA gene, partial sequence	994	994	96%	0.0	93%	KR025524.1

Figure 2 : Phylogenetic tree for *Avitellina* sp. using partial 18s rRNA gene sequence

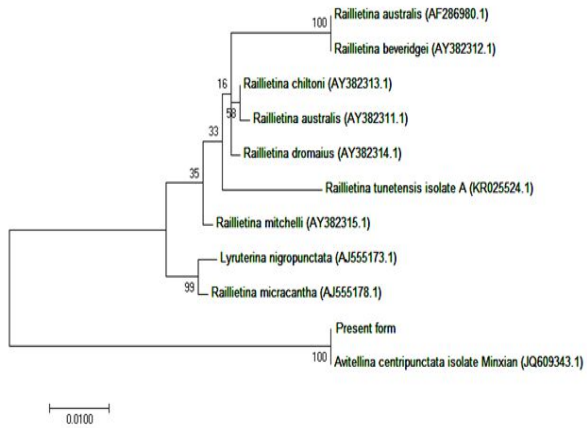
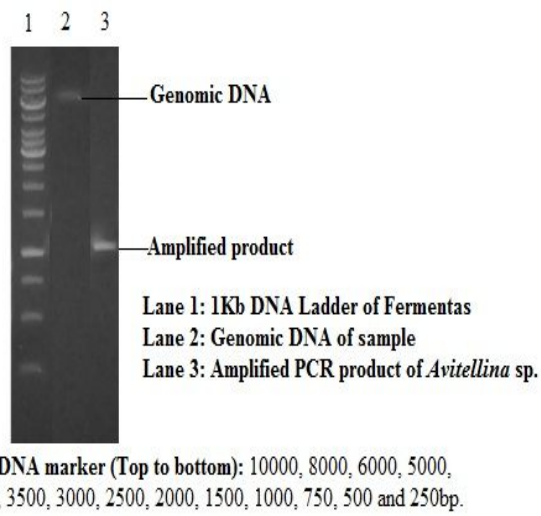


Figure 3: Genomic DNA extracted from worms



1Kb DNA marker (Top to bottom): 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250bp.

Molecular data:- A comparison of the partial sequences of the 18s rRNA gene of the present Cestodes with those of other Cestodes, in a phylogenetic context, provided further support for placing this species as a rediscription within *Avitellina centripanctata* thus confirming taxonomic conclusion based on morphological data.

In the phylogenetic trees (Fig. 2) obtained by maximum parsimony analysis of the 18s rRNA sequence data set, a close to the species *Avitellina centripanctata* is clear with a maximum identity 100% (Table no.1). After partial 18s rRNA gene sequence of *Avitellina* sp. sample DNA sequences length is ~1000bp.

DISCUSSION

The genus *Avitellian* was established by Gough, 1911 and as its type species *A. centripunctata* erected by Rivolta, 1874 in ovies aries. Later on four species were added to this genus (Woodland, 1927) i.e. *A. chalmeri* from *Ovis aries, A. goughi* from *Ovis aries*, *Bos taurus* and

Capra hircus, *A. lahorea* from *Bubalus bubalis* and *A. sudanea* from *Ovis aries* and *Capra hircus*. Then *A. sandgroundi* (Woodland, 1928) from *Hippotragus equines*, Later on *A. tatia* added from *Capra hircus* and *A. woodlandi* from *Ovis aries* (Bhalerao, 1936). Then *A. nagbhushanmi* from *Bos indicus* (G. B. Shinde, 1983). Recently *A. hircusae* from *Capra hircus* (Kale, 2005) *A. singhii* from *Capra hircus* (Shinde S.M., 2013) and *A. ali* From *Capra hircus* (Suryawanshi, 2015). The present form collected from *Capra hircus* in Jalna dist. (M.S) India.

The cestode under discussion comes closer to *Avitellina centripunctata* (Rivolta, 1874) in having all the essential morphological characters i.e. scolex is large in size distinctly marked, broader anteriorly and narrow posteriorly. Four suckers, neck long, mature proglottids broader than long, testes arranged in four fields, but differs from it in the following character i.e. It was collected from the host *Ovis aries* whereas present form found in the intestine of *Capra hircus,* testes number 14 to 16 in number Vs 16 to 18 in number, scolex Quadrangular Vs Rounded and par-uterine organ sac like Vs simple.

After discussion we concluded that the morphological observations clearly demonstrate that present form should be considered to be a member of genus *Avitellina* (Eucestoda- *Thysanosomatinae*) and the species having similar with *Avitellina centripunctata* Rivolta, 1874.
In molecular analysis the phylogenetic neighbors of *Avitellina* based on partial 18s rRNA gene are shown in table no. 1 the sample showed maximum similarity (100%) with the *Avitellina centripunctata* Rivolta, 1874.

As both morphological and molecular observations shows the characters of present form comes closer to *Avitellina centripunctata* (Rivolta, 1874). Therefore, it is redescribed here as *Avitellina centripunctata* Rivolta, 1874.

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