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MORPHOMETRIC AND MOLECULAR DIAGNOSIS OF TAPEWORM *RAILLIETINA* SPP. IN BRAHMAPOOTRA CHICKENS

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ABSTRACT

In this study, tapeworms of the genus *Raillietina* were diagnosed in Brahamas chickens in Mosul city, which suffered from emaciation and weakness, as some tapeworms were excreted in the chicken's feces. The mature segments of these worms stained with carmine appeared red in color and contained one set of reproductive organs and a regular unilateral genital opening. Both the ovary and the vitelline glands formed a ring-like shape located in the middle of the body segments. Gravid segments were distinguished by containing egg capsules that contain a number of eggs. The diagnosis of these worms was confirmed molecularly using the polymerase chain reaction technique based on 18srRNA and ITS genes, as well as with a molecular weight of 500 bp and 800 bp. Genetic sequencing results showed the diagnosis of two new isolates: *Raillietina tunetensis* SNEM1 gene for 18S rRNA of the partial sequence (LC764447.1) and *Raillietina sonini* SNEM2 gene for 18S rRNA of the partial sequence (LC764448.1). A phylogenetic tree recorded isolates based on a program Mega 11, as the ratio of the genetic proximity of the isolates to the *Raillietina echinobothrida* China isolate was 1.439. It was very far from the strain *Raillietina tunetensis* by 0.214.

Key words: *Raillietina* spp., Brahama chickens, PCR, phylogenetic tree

INTRODUCTION

Production of poultry has a great impact on the economy worldwide (Qui, 2023; Ahmed and Albakri, 2021; Saied and Hameed, 2023). It has been found that poor management affects poultry through several parasitic diseases (Abdulah and Mousa, 2023; Fadel and Mustafa, 2023; Al-Ali *et al.*, 2023). Cestodes parasitic infection, belongs to the family Davainieidae genus: and *Raillietina* is the most common tape worm infection in chicken (Chen and Li, 2014; Abdelqader *et al.*, 2008; Anwar *et al*., 1991). Three species of *Raillietina* occur in small intestine of fowl: *R echinobothridia, R. tetragona,* and *R. cesticillus,* in different parts of the world (Catelli *et al*., 1999; Butboonchoo *et al.*, 2016; Begum *et al.*, 2019; Alenyorege *et al*., 2011). Chickens become infected with *Raillietina* when ingested the intermediate host such as ants or

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beetles which contain the larval stage, cysticercoid (Bashini *et al.*, 2017).

Chickens infected with *Raillietina* show different clinical signs include loss of appetite, emaciation, diarrhea, digestive tract obstruction and decreased egg production (Al-Quraishy *et al.*, 2019).

Morphological characteristics in diagnosing species of *Raillietina* spp. depend on the shape and size of the head (scolex), rostellum, suckers, and position of genital pores and number of eggs in egg capsules in gravid segments (Catelli *et al*., 1999, Zhang *et al.*, 2021). However, morphological analysis may be confusing, and therefore, it is necessary to combine it with molecular methods for accurate and effective classification of *Raillietina* spp. (Borkowski *et al.*, 2020).

Therefore, this study was designed for the purpose of diagnosing *Raillietina* in Brahma chickens using both morphological and molecular diagnosis based on 18SRNA gene and internal transcribed spacer (ITS2), in addition to studying the gene sequences and phylogenetic tree.

MATERIALS AND METHODS

Samples Collection

A total of 30 chickens that were suffering from emaciation and severe diarrhea from some regions of Mosul city, tapeworms were collected from 10 brahama chickens. These tapeworms were collected directly from the feces of chickens.

The worms were kept in a warm phosphate buffer solution, the shape of those tapeworms was studied microscopically. These worms were divided into two parts, the first part of worms, especially mature proglotides were placed in 70% ethyl-alcohol and stained by carmine dye (Suleiman *et al.*, 2022), while the second parts of worms were kept at -80C° for PCR analysis.

Deoxyribonucleic acid (DNA) extraction

The DNA extracted from Raillietina by using a DNA extraction kit (Geneaid ®) according to the instructions described by the manufacturer. Then, the rehydration solution was added to the extracted DNA samples and preserved in -20°C until further test.

PCR Method

Molecular diagnosis of these tapeworms was done through the amplification of the:

1-Region 18sRNA and the PCR to diagnose *Raillietina*, depending on the following primers (Borkowski *et al.*, 2020)
Forward: 5′-AAGCCATGCATGTCTCAG TTCAG-3′
Reverse: 5′-GCCCTCCAATTGATCC TCGTG-3′.

The reaction mixture of the PCR (20µl) included: 10µl of Master Mix (Promega 2X), 1µl of each primer, 4µl of DNA template and 4µl of PCR grade water. This reaction was accomplished using a thermocycler (Optimum 96 G Germany R), and PCR cycles shown in (Table 1) (Foronda *et al*., 2005).

Table 1: Cycling condition for amplification of *Raillietina*

Stages	Temp. C°	Time	Cycle number
initial denaturation	95	6 min.	1
denaturation	95	45 sec.	35
annealing	55	1.0 min.	
extension	72	1.0 min.	
final extension	72	5 min.	1

2- ITS 2 region and PCR were done to diagnose *Raillietina* using the following primers (Butboonchoo *et al*., 2016):
Forward: 5´-GGT ACC GGT GGA TCA CTC GGC TCG TG-3´´
Reverse: 5´-TAT GCT TAA ATT CAG CGG GT-3.

PCR reaction mixtures prepared in 20µl containing 10µl Master mix (Promega 2X), 1µl of each primer, 4µl of DNA template and 4µl of PCR grade water. PCR was done using a thermocycler (Optimum 96 G Germany ®) and PCR cycles

Table 2: Cycling conditions of PCR for amplification of *Raillietina*

Stage	Temp. C°	Time	Cycle number
initial denaturation	94	2 min.	1
denaturation	94	45 sec.	35
annealing	57	1. min.	
extension	72	1. min.	
final extension	72	5 min.	1

The products of amplification were separated by electrophoresis in 2% agarose gel stained by a 4µl red safe. A 4µl of each PCR product was injected into the hole of agarose gel. The electrophoresis was run at 60 V for 60-70 min using a power supply containing 1X TBE buffer. The 100 bp DNA marker (Biolaps), 4 µl, was used as a standard molecular marker while the gel was examined under UV light (Gel Documentation).

DNA sequencing

The genetic sequences of *Raillietina* analyzed by using the Genetic Analyzer 3130 (Hitachi, Japan ®) and compared with NCBI

RESULTS

A large number of tapeworms were collected directly from feces of 10 brahama chickens, out of 30 chickens that were suffering from emaciation and severe diarrhea (Fig 1)

Figure 1: A large number of tapeworms excreted in chicken feces

After microscopic examination of the samples of worms dyed with carmine stain, mature proglotides were consisted of one set of reproductive organs with unilateral genital opening. Ovaries and vitelline glands showed ring shape, and the testes diffuse in the middle portion of the segments (Figure 2) and the eggs are found in egg capsules. (Figure 3)

Figure 2: mature segments of Raillietina species 10x by using digital camera.

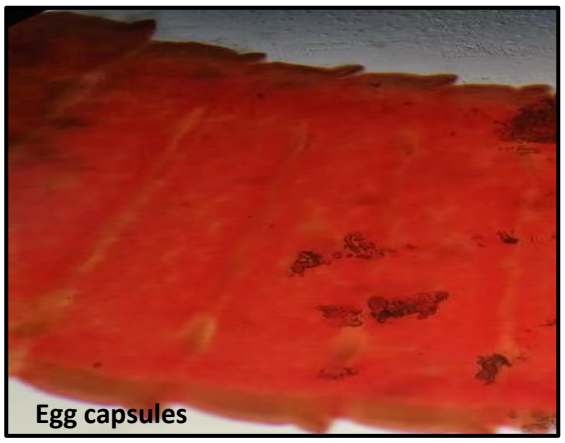

Figure 3: gravid segments of Raillietina species 10x by using digital camera

DNA extracted from some portion of tapeworms in 25 ng/µl. concentration. The extracted DNA concentration was 55-127ng and a purity of 1.8.

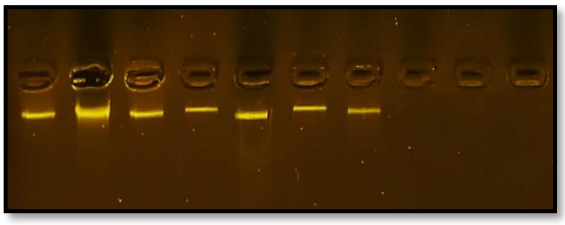

Figure (4): Bands of DNA extracted from *Raillietina* species migrated into 2% agarose gel.

The results of PCR indicated that the samples of DNA were used in this reaction might be used to confirm diagnosis *Raillietina* species based on both the genomic region18srRNA and ITS and amplification products, were 500bp and 800bp, respectively (Figure 5,6).

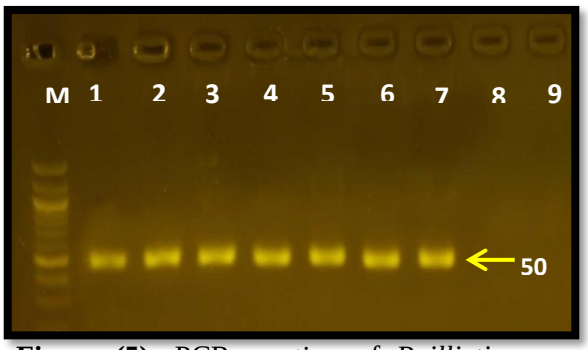

Figure (5): PCR reaction of *Raillietina spp*. based on the 18srRNA region and a amplification product of 500 bp.

Figure (6): PCR reaction of *Raillietina* spp. depending on the ITS region, and a amplification product of 800 bp.

The sequencing results revealed that the new isolates of *Raillietina from brahama chickens* in Mosul city; were recorded in Gene Bank, they were:

Raillietina tunetensis SNEM1 gene for 18S rRNA partial sequence GenBank: LC764447.1 and Raillietina sonini SNEM2 gene for 18S rRNA, partial sequence GenBank: LC764448.1 with accession number EU665465, EU665468.1 respectively (Figure 7,8)

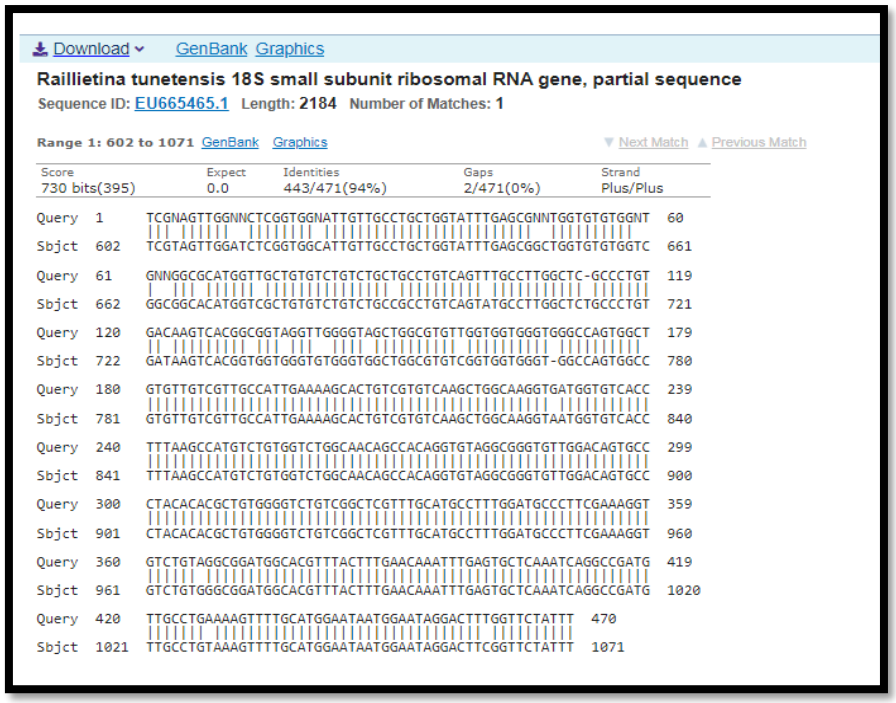

Figure (7): Taxonomic symbol of strain *Raillietina tuetensis*

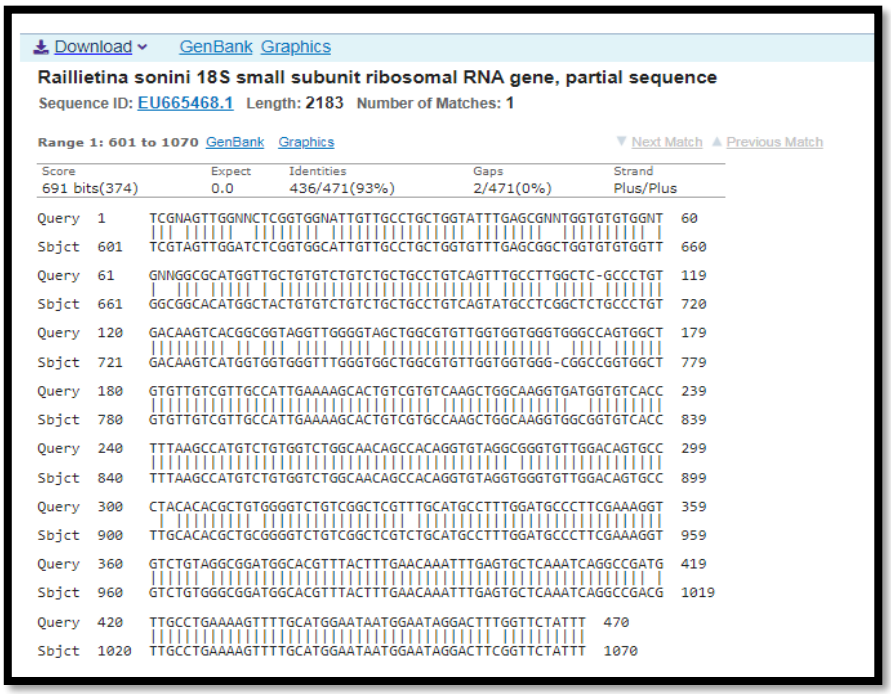

Figure (8): Taxonomic symbol of strain *Raillietina sonini*

A Phylogenetic tree was also prepared for the recorded isolates based on a program Mega 11, as the ratio of the genetic proximity of the isolates to the *Raillietina echinobothrida* China isolate was 1.439 It was very far from the strain *Raillietina tunetensis* by 0.214 (Figure 9).

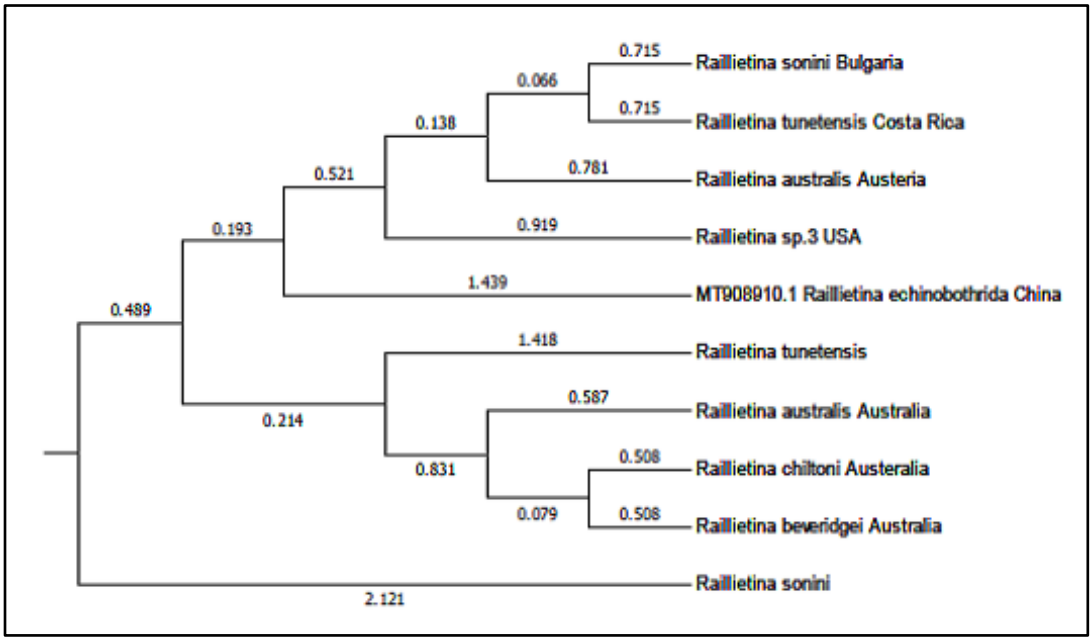

Figure (9): Phylogenetic-tree analysis of Raillietina species

DISCUSSION

In this study, genus *Raillietina* was diagnosed according to the morphological features of mature segments, which were characterized by the presence of unilateral opening of the genital pore. The gravid segment contains numbers of eggs sac that contain numbers of eggs, but it is difficult to determine the species of *Raillietina* depending on the morphology due to the differences among species (Zhang *et al.*, 2021; Butboonchoo *et al*., 2016).

Molecular methods are effective and accurate in identification of species and determination of genetic variation among population. Fewer information on the molecular diagnosis of *Raillietina* spp. are available (Alenyorege *et al*., 2011).

The PCR was performed in this study for the amplification of the specific 18 s rRNA gene (500bp) and ITS_2 gene (800bp) to confirm the diagnosis the Raillietina. By sequencing analysis, two species of *Raillietina* were diagnosed in brahama chickens in Mosul city; *Raillietina tunetensis* and *R.sonini*. The 18s rRNA and ITS.2 genes have been widely used for PCR detection and phylogenetic relationship study of parasite (Borkwski *et al*., 2020; Tanaka *et al*., 2014; Panich and Chontanorark, 2021). The species *Raillietina tunetensis and R.sonini* were reported for the first time in brahama chickens in Mosul city, Iraq. This result was in agreement with Makwanise *et al., (2020),* who recorded *R. tunetensis* in chickens in Zimbabwe. Also, Zhang *et al*., (2021) showed both 18s rRNA and ITS-2 genes were used commonly and successfully for the molecular diagnoses of *R.echinobothrida* in China.

Phylogenetic tree showed that MT908910.1 *Raillietina echinobothrida* China very far from the *Raillietina* with 1.418 and the clade of *Raillietina sonini* with 2.121. Butboonchoo *et al*., (2016) reported sequence information of ITS2 and NDI genes with a study of morphological and biometrical characteristics can be useful methods for clarification the relationships among the species in *Raillietina* group. Other studies in Africa revealed that the most host for Raillietina species are domesticated poultry (chickens, pigeons, turkeys, ducks and geese) (Nagwa *et al*., 2014). Makwanis *et al*., (2020)

recorded the *Raillietina tunetensis* is the predominant tapeworm in *Gallus gallus domesticus* from Zimbabwe. Littlewood *et al*., (2008) showed that the relationship within *Raillietina* spp. clades were unexplained with nuclear information but were far better explained by mitochondrial.

CONCLUSION

Tapeworms of the genus *Raillietina* were diagnosed in Brahamas chickens in Mosul city for the first time and *Raillietina spp.* are the most common worm's infection in chicken. By genetic sequencing we diagnose two new isolates: *Raillietina tunetensis* SNEM1 gene for 18S rRNA of the partial sequence (LC764447.1) and *Raillietina sonini* SNEM2 gene for 18S rRNA of the partial sequence (LC764448.1).

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تشخيص شكلي وجزيئي للديدان الشريطية *Raillietina* spp. في دجاج البراهاما

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تم في هذه الدراسة تشخيص الديدان الشريطية من جنس الريليتينيا في دجاج البراهاما في مدينة الموصل، والتي كانت تعاني من الهزال والضعف، اذ لوحظ طرح أعداد كبيرة من الديدان الشريطية في براز الدجاج. ظهرت قطع الديدان الناضجة والمصبوغة بصبغة الكارمين باللون الاحمر وتحتوي علي مجموعة واحدة من الاعضاء التناسلية وفتحة تناسلية احادية جانبية منتظمة وشكل كل من المبيض والغدد المحية شكل الحلقة والتي تقع في وسط القطع الجسمية وتميزت القطع الحوامل باحتوائها علي محافظ البيوض التي تحتوي علي عدد من البيوض. تم تأكيد تشخيص هذه الديدان جزيئياً باستخدام تقنية تفاعل البلمرة المتسلسل المعتمدة على جينات الحامض النووي الرايبوزي ١٨ و جين فواصل النسخ الداخلية، وكانت بوزن جزيئي٥٠٠ زوجا قاعديا و٨٠٠ زوج قاعديا. أظهرت نتائج التسلسل الجيني تشخيص عزلتين جديدتين وهما الريليتينيا التويتنسيسLC764447.1 والريليتنيا سونيني LC764448.1 . سجلت شجرة النشوء والتطور عزلات بناءا على برنامج تحليل الوراثة التطورية الجزيئية ١١ إذ بلغت نسبة القرب الوراثي للعزلات من عزلة رايليتينا إشينوبوثريدا الصينية ١,٤٣٩ وكانت بعيدة جداً عن سلالة الريليتينيا التويتنسيس بمقدار ٠,٢١٤.

الكلمات المفتاحية: الريليتينيا ، دجاج البراهاما، التشخيص الجزيئي، الشجره الوراثيه