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First record of a new Sarcocystis species isolated by genetic analysis from beef meat in Baqubah, Iraq

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Article info

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Sarcocystosis is important disease in Asia, especially the western regions of the continent. It is considered one of the important zoonotic diseases, the infection wide geographic spreading caused by various species. in Iraq the disease is cosmopolitan infected man and animals leading to public health disorders and economic losses in meat production. the study was made to demonstrate the Sarcocystis species from local beef meat in Diyala provinces. Sarcocysts isolated from esophagus of 100 cattle were slaughtered at Diyala slaughter house during the period from November 2023 to May 2024. Cattle age ranged from 2–5 years. Different traditional techniques was made to detected sarcocystosis, peptic digestion technique were high sensitive technique 100 % methods. Then Trichnoscopy were (30 %) while the squeezing less sensitive (26 %) respectively. The majority of male animals recorded higher infection incidence 75 % while the females cattle rate was 53.8 % from the total exanimated animals. The result showed that the highest infection rate were recorded in animal of old age above 4 year old 100 % with significant differences between the age groups. The bradyzoites of *Sarcocystis* parasite were seen by examining the sediment of the digested muscle fluid as banana shape with a spik end of front and rounded rear end and slightly clear nucleus positioned toward the rear end,measurements 13.2 × 2.8 µm. The study was depending on using a traditional thermocycler PCR, as well as Sequencing, genotyping analysis of the Sarcocystis isolated from Diyala (Baqubah), Iraq. Extracted and isolate of genomic DNA of Sarcocystis, depending on Gene aid, obtained from Tissue cells extraction by using peptic digestion technique according to manufacturer protocol. Primer amplified using fragment over 900 bp. The purity of DNA was demonstrated by Nanodrop spectrophotometer at range (1.6–1.8 ng/µL), *Sarcocystis levinei* was detected for the first time in Iraq. The genetic analysis showed that the Iraqi isolates of *Sarcocystis cruzi* given a remarkable homology (100 %) with Norway, China, Iran, Turkey, India, Japan and Argentina respectively.

Keywords: Cattle,Genetic analysis, Iraq, Sarcocysts levinei.

Перший повідомлення про новий вид Sarcocystis, виділений генетичним аналізом з яловичини в Бакуба, Ірак

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Саркоцистоз є важливим захворюванням в Азії, особливо в західних регіонах континенту. Збудники саркоцистозу проявляють виражений зоонозний потенціал, а отже захворювання на саркоцистоз віднесене до списку важливих зоонозних хвороб. Відомо, що хворобу здатні викликати різні види саркоцист, які географічно достатньо широко розповсюджені по всьому світу. В умовах Іраку саркоцистоз є надзвичайно поширеним як серед тварин, так і серед людей. Слід зазначити, що значне ураження *Sarcocystis* spp. тварин має системний характер та призводить до колосальних економічних збитків виробникам м'яса в країні. Окрім того, значне поширення саркоцистозу серед тварин призводить до ураження ним людей, що чинить додаткове фінансове навантаження на систему громадського здоров'я. Метою здійсненого дослідження було продемонструвати циркулюючі у провінці Діяла (Бакубіа) види *Sarcocystis*, виявлені з яловичини. Біологічні зразки для досліджень (фрагменти м'язової тканини стравоходу від великої рогатої худоби віком від 2 до 5 років, n=100) були відібрані в умовах забійних пунктів Діяла (Бакуба) у період з листопада 2023 року по травень 2024 року. Для виявлення саркоцист були використані традиційні методики досліджень зразків. Зокрема, техніка перетравлювання у штучному шлунковому соку, трихінелоскопія та компресорне дослідження, діагностична ефективність яких у описаному дослідженні становила 100, 30 та 26 % відповідно. Дослідженнями встановлено, що ураженість тварин різної статі збудниками *Sarcocysts* spp. мала певні відмінності. Зокрема, рівень інвазованості серед самців складала 75 % від загальної кількості обстежених тварин, тоді як у самок цей показник був на рівні 53,8 %. У віковому аспекті найвищий рівень інвазованості *Sarcocystis* spp. зафіксовано серед тварин старше 4 річного віку (100 %). За використання класичного методу дослідження зразків (перетравленням у штучному шлунковому соку) було виявлено брадизоїти *Sarcocystis* spp., які мали бананоподібну форму з гострим переднім та округлим заднім кінцями, і злегка прозорим ядром, що було розташоване у напрямку до заднього кінця. Розміри виявлених інвазійних елементів становили 13,2 × 2,8 мкм. Окрім того, були проведені дослідженим біологічного матеріалу з використанням традиційного термоциклера ПЛР з послідуючим аналізом генотипу *Sarcocystis*. Вперше виявлено на території Іраку наявність виду *Sarcocystis levinei*. Окрім того, встановлено, що іракські ізоляти *Sarcocystis cruzi* мають високу гомологію (100 %) з ізолятами, що виявлені на території Норвегії, Китаю, Ірану, Туреччини, Індії, Японії та Аргентини.

Ключові слова: велика рогата худоба, генетичний аналіз, Ірак, *Sarcocysts levinei*.

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Introduction

The worldwide illness sarcocystosis affects both herbivores (intermediate hosts) and carnivores (definitive hosts). Sarcocytosis is prevalent in Iraq; the disease has a high seasonal infection rate that reduces the amount of meat cattle and other domestic ruminant animals can produce [1–4].

The identification of Sarcocystis can be done in a number of ways, such as visual inspection of the meat, microscopic inspection of the infected muscle, quash, meat digestion [5], immunofluorescence test, histopathology examination, polymerase chain reaction (PCR), and other PCR tests like multiplex PCR and nPCR [6–8]. Four common genes (mitochondrial COX1, 28s rRNA, 18s rRNA, and ITS-1) are utilized to identify species of Sarcocystis [9–11]. A 28s rRNA and 18s rRNA are highly conserved and more helpful for intraspecific identification. Mitochondrial COX1 and ITS-1 have also been found to have strong specificity, particularly for interspecies (sub species) identification [12, 13].

Because the 18S rRNA gene region's hyper-variability regions are scattered with conserved DNA sequences, it can be used to distinguish between the apicomplexan group and the genus Sarcocystis from other ccocidain protozoa, making it a more technically accurate marker than the mitochondrial cox1 sequence [14–15].

Additionally, the databases include the Sarcocystis parasite's 18S rRNA sequences, which are prepared for use in species identification with other molecular gene sequences [16]. The (18S rRNA) gene is therefore a useful diagnostic marker because of these features [17, 18].

The aim of the study

The current study was designed to employ molecular recognition of the Sarcocystis species infecting beef meat in Diyala province (Baqubah), Iraq.

This was done by amplifying the 18s rRNA region, which virtually amplifies at the 829 bp segment (Sar) by using the primer BLAST tool on the NCBI Gene Bank.

Materials and methods

Collection of Samples: one hundred meat Specimens were obtained from cattle that slaughtered at Diyala slaughter house during the period from November 2023 to May 2024. Cattle age ranged from (2–5) years.

Gross Examination: muscles of Esophagus, diaphragm, were examined visually to detect sarcocysts tissue cysts. After that sarcocysts parasite were carefully isolated from infected muscle and then transferred in to plastic labeled bags and conveyed to Styrofoam box. In a timely manner the beef meat samples transfer to the Parasitology laboratory in collage of Veterinary medicine, University of Diyala, Iraq.

DNA Extraction: extracted and isolate of genomic DNA of *Sarcocystis*, by using Gene aid, obtained from Tissue cells extraction by using peptic digestion technique according to manufacturer protocol.

Primers: two different types of primers were employed in current work to identify *Sarcocystis* spp. isolates based on 18S rRNA (***Fig. 1***). The two lyophilized primers, which almost amplify at the 829 bp segment (Sar), were sent to IDT Company (Canada) using the primer BLAST tool on the NCBI of the Gene bank.Preparation of primers is performed as described primer fragment over (900 bp) was amplified using a primer:

forward primer GATAACCGTGGTAATTCTATG

reverse primer GGCAAATGCTTTCGCAGTA

Products 829bp, which is capable of detection of *Sarcocysts* spp. (***Fig. 2***).

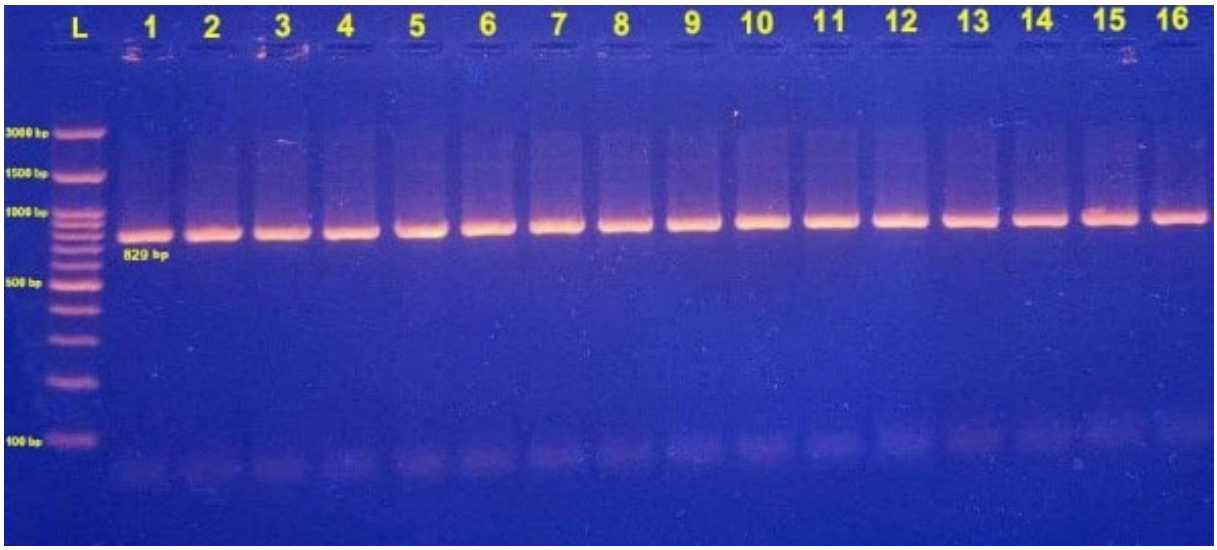

Figure 1. Electrophoresis of the 18S rRNA gene used for PCR assay for the local tested isolates of *S. cruzi*. Lane M 100-bp ladder = DNA marker. Lanes 1-16 represent the positive diagnostic bands on 829 bp. of the tested specimens

DNA concentration measurement: the purity of DNA was demonstrated by Nanodrop spectrophotometer.

Genetic analysis and sequencing of an Iraqi Sarcocystis isolate: In accordance with the sequencing company's instruction manuals, the PCR amplicons were commercially sequenced from termini, forward, and reverse primer (Macrogen Inc., South Korea). Applied Biosystem Extension (ABI) provides a clear chromatograph. In order to ensure that the annotation and diversity are not the result of PCR or sequencing artifacts, the sequencing data were also examined. The precise position and other details of the recovered PCR fragments were determined by comparing the observed DNA sequences of the local isolates from Iraq with the reference DNA sequences of *Sarcocystis* spp.

Interpretation of sequencing data: Bio Edit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA),was used for edited, aligned, and analyzed results of sequencing the PCR products referred to different local isolate so long as with the respective sequences in the database references. The results of genetic analysis showed that the Iraqi isolates PQ156402, PQ156403, PQ156404 have a 100 % relationship with Norway (KU247922.1), China (OR553291.1, MH681972.1), Iran (KR136315.1), Turkey (MF327255.1), India (KT306827.1), Japan (LC171828.1), Argentina (X679468.1). As shown in the (***Fig. 2***).

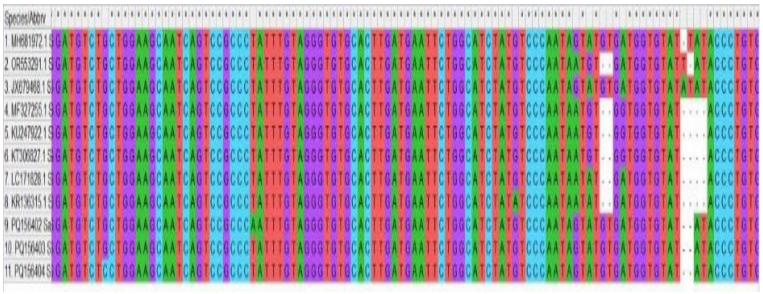

Figure 2. Polymerase (PCR) test results for (Sarcocystis).
A remarkable similarity in the primary sequence alignment was discovered in the.18s rRNA region for local tested isolates of cattle *Sarcocystis cruzi* (GenBank accession number PQ156402), registered in Genbank-NCBI

Ethical Statement our study is not based on any vivo experiments or live cattle; the sampling was implemented after the slaughtering of cattle in the slaughterhouse.

Results and discussion

Moleculer detection of Sarcocystis:

DNA Extraction and PCR amplifications: the diagnostic DNA to be isolated as compact bands illustrate the amplified target gene of Sarcocystis isolated from slaughter animals by using a traditional thermocycler PCR. Genomic DNA was extracted using according to manufacturer's instructions. (Macrogen Inc., South. Korea). After DNA extraction, the samples were stored at -20^{O}C until PCR preparation process is completed. Two regions of ribosomal RNA (18s-rRNA) gene have been targeted and separately well be amplified for detection *Sarcocystis cruzi*. A specific primer was amplified by used fragment over 900 bp. the Products 829 bp, which is qualified for detection of *S. levinei* (***Fig. 1***). The results of genetic analysis showed that the Iraqi isolates PQ156402, PQ156403, PQ156404 have a 100 % relationship with Norway (KU247922.1), China (OR553291.1, MH681972.1), Iran (KR136315.1), Turkey (MF327255.1), India (KT306827.1), Japan (LC171828.1), Argentina (X679468.1). As shown in the (***Figure 3***) and (***Table 1***)

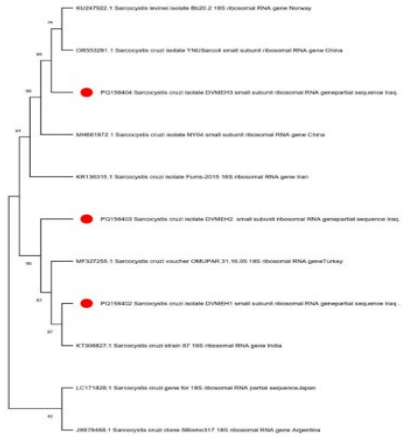

Figure 3. Phylogenetic tree analysis of *Sarcocystis* species based on the 18S rRNA gene sequence constructed according to Specific homology to local isolates.
The branch lengths are indicated the amounts of identity between the local strain and global strain

Table 1
Specific homology (Identity) of local *Sarcocysts* species in cattle

Accession no.	Country	Gene	Source	Compact.
PQ156402	Iraq	18S ribosomal RNA	*Sarcocystis levinei*	100%
PQ156403	Iraq	18S ribosomal RNA	*Sarcocystis cruzi*	100%
PQ156404	Iraq	18S ribosomal RNA	*Sarcocystis cruzi*	100%
KU247922.1	Norway	18S ribosomal RNA	*Sarcocystis cruzi*	100%
OR553291.1,	China	18S ribosomal RNA	*Sarcocystis cruzi*	100%
MH681972.1	China	18S ribosomal RNA	*Sarcocystis cruzi*	100%
(KR136315.1)	Iran	18S ribosomal RNA	*Sarcocystis cruzi*	100%
MF327255.1	Turkey	18S ribosomal RNA	*Sarcocystis cruzi*	100%
KT306827.1	India	18S ribosomal RNA	*Sarcocystis cruzi*	100%
LC171828.1	Japan	18S ribosomal RNA	*Sarcocystis cruzi*	100%
X679468.1	Argentina	18S ribosomal RNA	*Sarcocystis cruzi*	100%

Sarcocystosis is still a significant disease in Iraq which they infect man and several domesticated animals (cattle, sheep, goat, buffalo, etc.) causing serious economic effects on cattle herds [19–22].

Our own results indicated that detected DNA of coccidian parasites (Sarcocystis) belonging to the Apicomplexa protozoa parasite.as a molecular technique that could be identifying subspecies. Another previous studies in molecular and biological assay well be improving many genetic analyses of sarcocystis parasites [11, 23–25].

The molecular study detected two regions of ribosomal RNA (18s-rRNA) gene have been targeted and separately well be amplified for detection Sarcocystis cruzi, A specific primer was amplified by used fragment over 900bp. The Products 829bp, which is qualified for detection of *S. levinei* [26], various molecular studies and phylogenetic tree analyses have been progressing to distinguish *Sarcocystis* spp. including, the ITS-1 region, 18S rRNA, 28S rRNA [2, 8, 17, 27]. The alterable regions of the 18S rRNA gene act as advantageous targets for detecting and identifying different species [3, 9, 19, 28, 29].

The Sequence study and genotyping (phylogenic analysis) of *Sarcocystis* spp. Isolate from Iraq recorded a new strain of Iraqi *Sarcocystis* spp. (*Sarcocystis levinei* PQ156402) on Diyala city areas, the results of Phylogenetic tree analysis were clustered with the *S. cruzi*. Which given a remarkable homology with Iraqi isolates PQ156402, PQ156403, PQ156404 have a 100 % relationship with Norway, China, Iran, Turkey, India, Japan and Argentina respectively. the accordance of Sequencing and genotyping of *Sarcocystis* Isolate recorded some identity with other Previous studies in Iraq [16, 20, 30–33]. Further studies should be done in other Iraq provinces to identify or detection other *Sarcocystis* species in cattle which may be help in understanding the clinical diseases and economic losses resulting from the infection [34, 35].

Conclusions

The current study proven a new strain of Iraqi *Sarcocystis* spp. (*Sarcocystis levinei* PQ156402) in Diyala provinces, *Sarcocystis cruzi* in cattle was cosmopolitan in distribution , increased the import of cattle from turkey, India, South America to Iraq led to elevated the identity between the Iraq strain and other countries.

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Authors' contributions

Enas Nazar Abood and Haleem Hamza Hussain Al- Zubaidei:sampling; analysis of results; PCR; writing the manuscript; and editing the final version of manuscript.

Conflict of interest

The authors state that there is no conflict of interest.

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