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Detection of Sarcocystosis in Dogs in Nineveh Province

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Abstract

According to the knowledge, no prior genetic research had identified the *Sarcocystis spp.* in the definitive host (dogs) in Nineveh province, Iraq. Therefore, the goal of this work was to detect Sarcocystis species in dog feces targeting 18S rRNA gene amplifications. A total of 63 fecal samples from 63 stray dogs randomly chosen from various parts of Nineveh province, Iraq, between April and September 2023, aged (≥8 months) and sex (male=37, female=26), were collected in sterile plastic containers. Two hundred microliters of saline solution are added to 1 gram of feces for each sample to form a fecal suspension for DNA isolation and then Conventional Polymerase chain reaction (cPCR)is performed. The finding showed that out of 63 fecal samples detected by cPCR technique. 4/63(6.34%) of the samples tested positive in dogs, 3 female 11.53% and 1 male 2.7% respectively. Gel electrophoresis for Sarcocystis spp. DNA, showed that the positive bands were approximately 900 bp in Nineveh province for the first time. This work highlights the value of genetic testing for identifying Sarcocystis species and offers an invaluable diagnostic resource for future epidemiological research and the evaluation of the efficacy of this disease's management strategies.

Keywords: Sarcocystosis, c-PCR technique, Prevalence, Dogs, Nineveh - Iraq

Introduction

According to Marandykina *et al.* [1], *Sarcocystis spp.* are obligate intracellular parasites that infect humans as well as domesticated and wild animals. The life cycle of these genus is required prey-predator host [2]. The sporocysts grow in the intestine of the final host after consuming mostly muscular tissues containing mature Sarcocystis, and are predominately generated in the muscles of the intermediate host, which acquires the infection through contaminated food or water [3,4].

Both in the final and intermediate hosts, respectively, are where the life cycle takes place. Sarcocysts are generated in the cardiac and skeletal muscles of the intermediate host after going through a number of developmental phases [5,6]. Canids represent an important animal that acts as a host and reservoir for different parasites of concern to humans and livestock through the liberation of eggs, oocysts, and larvae, leading to consequences diseases and serious complexity [7].

On the basis of parasitological and phenotypic analysis, the domestic dog is the ultimate prevalent and public definitive host of diverse Sarcocystis species, according to the literature of Dubey *et al.* [8]. It has also recently been found that dogs are an intermediate host for *S. caninum and S. svanai.* Even though *Sarcocystis spp.* rarely poses a threat to the carnivores, diarrhea is still a possibility. In contrast, they often experience substantial tissue damage in the herbivorous intermediate host, which results in higher mortality and monetary losses [9]. Currently over 200 species of Sarcocystis are recognized, but their numbers have been steadily rising. and only molecular methods can distinguish between the species in their hosts [6,10].

According to the most recent global revisions [11,12], the estimated spread of

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Sarcocystosis in dogs ranged 2.2 to 9%. Low occurrence (0.3%) was detected in dogs [13]. Though some studies [14,15] recorded a higher rate in domestic dogs and sheepdogs in Ethiopia, with percentages 42-72% and 28.5%, respectively. These pronounced differences could be explained by a number of variables, including geography, management, and the types of laboratory methods. [16]. Dogs are also considerably risky to an infected animal, especially in the vicinity of slaughterhouses where aborted fetuses, visceral organs, and placentas of herbivorous animals are freely available. As a result, dogs might become at hazard of developing infections with parasites such as *Sarcocystis spp.* [17].

Presently, Sarcocystosis is diagnosed using a variety of conventional methods such as trichinoscopy, methylene blue staining, dob-smear, digestion, and histology. These techniques can only be used on slaughtered carcasses and are genus-specific. Additionally, a number of tests, such as the immunofluorescence antibody test (IFAT) and Enzyme-linked immunosorbent assay (ELISA), have been used to diagnose infections recently. However, because different *Sarcocystis spp.* are cross-reactive, these methods have low sensitivity and specificity [18]. Furthermore, the use of molecular techniques, like the cPCR and restriction fragment length polymorphism, stands out as a crucial and accurate alternative method for identifying stages of *Sarcocystis spp.* [19]. Detection of *Sarcocystis spp.* was demonstrated to be more accurate with molecular approaches than with morphological techniques. Usually, during the post-mortem examination, *Sarcocystis spp.* are invisible by sight [20]. Furthermore, it can be challenging to use transmission electron microscopy and fresh microscopic examination techniques on large samples. Therefore, in-depth research on Sarcocystis infection using molecular techniques is needed [21,22]. The Molecular detection of Sarcocystis species in dogs has not been studied before in Nineveh province. Hence, the aim of this research was to genetically confirm the presence of Sarcocystis in the feces of dogs in Nineveh province, Iraq, and this will be useful for our future epidemiological research.

Material and Methods

Ethical approval

Ethically the work was done according to the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, University of Mosul (Um.VET.2023.023).

Animals and Samples collections

In this study, 63 fecal samples from 63stray dogs were randomly chosen from various parts of Nineveh province, Iraq, between April and September (2023), and they ranged in age (≥8 months) and sex (male=37, female=26). The fecal samples were collected in sterile plastic containers. Two hundred microliters of saline are added to 1 g of feces for each sample to create a fecal suspension for DNA isolation. To break the oocyst walls, the obtained suspension was frozen and thawed three times and samples kept at −20 °C till use [23].

DNA extraction and PCR amplification

The Presto^{TM} Stool DNA Extraction Kit's Protocol Procedure was followed in the preparation and processing of the samples (Geneaid Biotech, Korea). With the PCR Kit (Bioneer, Korea), which targets 18S rRNA gene to detect *Sarcocystis spp*., manufacturer's instructions were followed to prepare the Master Mix tubes at a final volume of 20 µl. *Sarcocystis spp.* were identified using the primers Sar- F: (5-GGATAAACCGTGGTAATTCTATG-3) and Sar-R: (5-GGCAAATGCTTTCGCA GTAG-3) [24]. The Thermocycler System (BIO-RAD, USA) was used to perform a conventional PCR reaction. The reaction mixture contained 10 µl of 2X Master mix (Jena Bioscience) containing Taq buffer, and dNTPs, 6 µl free ionized water, 1 µl (10pmol) of each primer (Macrogean, Seoul, Korea) and 2 µl (200 ng) DNA sample. The cycling conditions of PCR were as follows: initial denaturation at 95˚C for 2 min, followed by 40 cycles of denaturation at 94˚C for 40 sec, annealing at 55˚C for 35 sec, extension at 72˚C for 1 min and final extension at 72˚C for 6 min. To analyze the PCR products, 1.5% agarose gel electrophoresis (100V, 80Am 1 hour). Using a UV illuminator (Clinx Science, China) and a digital camera (Nikon, Japan), the amplified DNAs' final product sizes were then observed and recorded. According to Imre *et al.* [24], 900 bp samples were predicted to be positive for Sarcocystis spp.

Statistical analysis

Descriptive statistics in the Excel program 2010 was used to calculate the prevalence.

Results

Out of 63 fecal samples of (male=37, female=26), detected by c-PCR technique,

4/63(6.34%) of the samples tested positive in dogs, 3 female 11.53% and 1 male 2.7% respectively (Table 1). The results for *Sarcocystis spp.* DNA, on gel electrophoresis showed that the positive bands were at nearly 900 bp (Fig. 1).

TABLE 1. Prevalence of *Sarcocystis spp.* in dogs using cPCR technique

Gender	No. of sample	No. of +ve (%)	No. of -ve (%)
Male	37	1 (2.7%)	36 (97.29%)
Female	26	3 (11.53%)	23 (88.46%)
Total	63	4 (6.34%)	59 (93.65%)

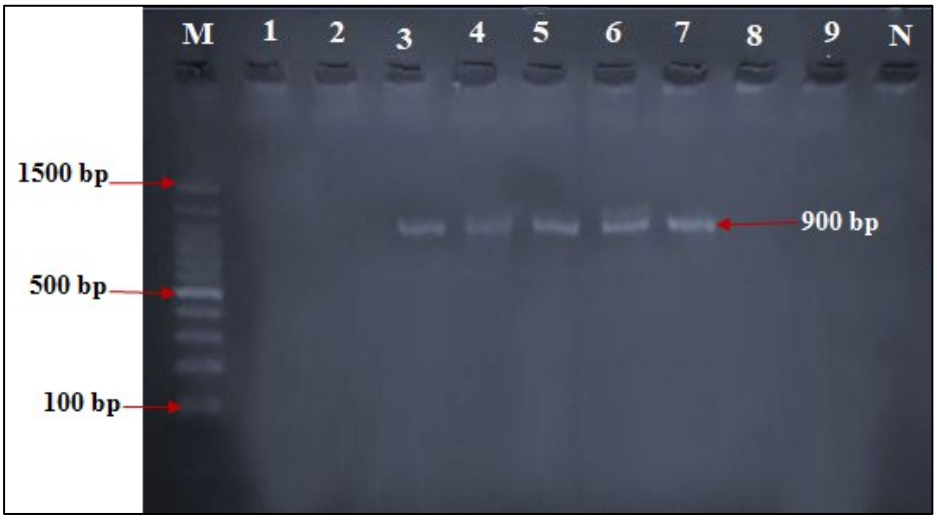

Fig. 1. Electrophoresis image lane M) 100-1500bp DNA ladder; Lanes 3-7) The positive dogs for *Sarcocystis spp.* in approximately band size 900bp; Lane N) negative control.

Discussion

It has been shown that *Sarcocystis spp.* were one of the widespread significant economic parasites of veterinary [6,25]. As there is no former molecular index been to detect Sarcocystis *spp.* in Nineveh province, Iraq. Our study was the initial molecular report in the domestic dogs. The prevalence of *Sarcocystis spp.* in dogs was 6.34% in Nineveh province through utilizing the molecular analysis. Previous literature documented various prevalence rates using diverse laboratory techniques in different countries. In Chile, was 4% [11]. In Nigeria was 9.0% [12]. In Ethiopia was 28.5% [26]. In Brazil was 2.2% [27]. The disparity between these finding could be due to several justifications such as regions, number of samples, demographics distribution, uses of antiparastic therapy, and laboratory tests. Our conclusion is in line with former studies (15,16). In general, and according to the international literatures the rate of sarcocystosis prevalence in dog ranged 2.2 - 9% [27]. In Iraq, several earlier researches recorded different rates of *Sarcocystis spp.* in small and large ruminants in different governorates. In Baghdad, Iraq, prevalence in the slaughtered sheep, goats, cattle, water buffaloes and camels were 4.1, 33.6, 0.2, 15.6 and 0 [28]. Alhayali *et al.* [29] recorded in a case report in one years old sheep. In Duhok, Iraq [30] was 16.77% and 13.62% in slaughtered sheep and goats respectively. In Al-Diwaniyah province, in sheep [22] demonstrated that 97% by PCR. The purpose for mention of these data in Iraq in small and large ruminants which are the intermediate hosts [20,31], for Sarcocystosis parasite was to assertion to an is deleted

important evidence for the role of the dogs as one of the finals hosted of this parasite. Our vision is in accordance with what mentioned by Dubey *et al.*, Rokni and El-Dakhly *et al.* [3,32,33]. It has been referenced that the definitive hosts are the key operator in the spread of Sarcocystosis, and in order to impede the circle, these carnivores must be prevented from ruminants feed, water. Also, the slaughtered or dead livestock carcasses have to never be fed or left in the field for dogs and cats [17]. Moreover, it has been revealed that dogs also could serve as an intermediate host with clinical muscular Sarcocystosis as announced by Dubey *et al.* [3]. Despite the parasite has minor effects to the final host, however chronic or sometime acute GIT disturbance may occur such as diarrhea may result, while these parasites in herbivorous intermediate host, may result in wide tissue damage and resultant excess mortality, inappetence, diarrhea, emaciation which can negatively affect meat quality and marketing and collectively lead to serious economic losses [30,34,35].

Successfully, the molecular outcome of the present work detects the DNA of *Sarcocystis spp.* in fecal samples of dogs which indicate sensitivity of this technique. This result similar to the conclusion of [20,21,36]. It is known that the molecular methods, are requisite to distinguish the species of this genus, which are unable to perform only by microscopy. And the PCR technique is very significant for species identification and epidemiological studies [37,38].

Conclusion

Based on the findings that have been mentioned former in this study, it was revealed that the *Sarcocystis spp.* are prevalent in the dogs (one of the important final hosts) in Mosul city, Iraq which represented a significant assistance in the epidemiology and prevalence of Sarcocystosis disease in farm animals in addition to the public concern. Additional studies require to investigate this species using different molecular targets, phylogenetic analysis and identification of species-specific parasites in dogs and other possible final hosts in Nineveh province.

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Conflict of interest

Authors state no conflict of interest found in this study

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الكشف عن الساركوسيستوسيس في الكلاب في محافظة نينوى

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الخلاصة

وفقا لمعرفتنا، لم تحدد أي أبحاث وراثية سابقة للساركوسيستيس. في المضيف النهائي (الكلاب) في محافظة نينوى بالعراق. لذلك، كان الهدف من هذا العمل هو الكشف عن الساركوسيستيس في براز الكلاب من خلال تضخيم جينات الرنا الرايبوسي 18S rRNA. تم جمع 63 عينة براز من 63 كلبًا ضالًا تم اختيارها عشوائيًا من أجزاء مختلفة من محافظة نينوى، العراق، في الفترة ما بين أبريل وسبتمبر 2023، بعمر (≤8 أشهر) والجنس (ذكر = 37، أنثى = 26). تم إضافة مائتي ميكروليتر من المحلول الملحي إلى 1 جرام من البراز لكل عينة لتكوين معلق برازي لعزل الحمض النووي لغرض إجراء تفاعل البلمرة المتسلسل التقليدي. أظهرت النتائج أنه من بين 63 عينة براز التي فحصت بتقنية تفاعل البلمرة المتسلسل التقليدي، كانت 63/4 (6.34%) من العينات إيجابية في الكلاب، منها 3 إناث 11.53% وذكر واحد 2.7% على التوالي. وأظهر الحمض النووي أن النطاقات الإيجابية كانت حوالي 900 زوج قاعدي في محافظة نينوى لأول مرة. يسلط هذا العمل الضوء على قيمة الاختبارات الجينية لتحديد أنواع الساركوسيستيس ويوفر موردا تشخيصيا لا يقدر بثمن للبحوث الوبائية المستقبلية وتقييم فعالية استراتيجيات إدارة هذا المرض.