Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Nc-5 gene-dependent molecular identification and phylogenetic investigation of *Neospora caninum* in sheep

A.C. Karawan and M.J. Alkhaled

Department of Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq

Article information

Article history:
Received 13 September, 2023
Accepted 02 December, 2023
Published online 15 March, 2024

Keywords:
Coccidia
Enteroparasites
Protozoa

Correspondence:
A.C. Karawan
azhar.karawan@qu.edu.iq

Abstract

The current study was conducted to identify the occurrence of *Neospora caninum* in sheep using molecular techniques. The investigation included 200 sheep samples (20 heart and 80 brain tissues and 100 blood) collected from different Al-Qadisiyah Province, Iraq districts. After extracting the DNA, the samples were subjected to molecular techniques, polymerase chain reaction (PCR), and partial gene sequencing accompanied by estimating the phylogenetic status of the *N. caninum* coccidial microorganism. Both techniques relied on detecting the *Nc-5* gene, a repetitive region of the organism's DNA. The finding of the PCR revealed the genetic identification of 7 (3.5%) isolates of *N. caninum* in the samples of the examined sheep. For the sequencing, seven PCR products were detected as *N. caninum* with nucleotide close similarity to isolates from some regions, such as New Zealand and Switzerland. The findings demonstrated by the herein study that sheep can be an intermediate host for *Neospora caninum*; however, all these global isolates were reported only from species other than from sheep; the current study target animals.

DOI: 10.33899/ijvs.2023.138333.3227,

Introduction

The apicomplexan protozoan, *N. caninum*, causes substantial economic losses for farmers and the animal production sector worldwide. Ruminants serve as intermediate hosts for the parasite throughout its two phases of development (tachyzoite and tissue cyst), whereas canines serve as final hosts. Herds may spread this pathogen both horizontally and vertically. Fetal abortion, stillbirth, or the delivery of an animal with no apparent signs of illness are all possible outcomes of placental infection (1-3). The major pathway of abortion produced by *N. caninum* is congenital transmission, which is why this parasite can live in farms and herds for years. There have been reports of spontaneous infections in other ruminants, such as sheep and goats, even though cattle are the most crucial host for *N. caninum*. Infection rates with *N. caninum* in sheep and goats vary widely between regions and nations (4-8), which have various seroprevalence rates, which may be due to climatic conditions, livestock nutrition, and health care practices (9-12). Published research findings and meta-analyses indicate the seroprevalence of *N. caninum* infections in sheep and goats to be 12.0 and 5.99%, respectively (13). On the other hand, goats are often browsers, whereas sheep spend most of their time grazing, making them more susceptible to infections from the ground (14). When small ruminants were experimentally inoculated with *N. caninum*, pregnant settings comparable to those in cows were generated. Due to a shortage of research, we still do not know the potential of neosporosis via its clinical, epidemiological, and economic impact on sheep and goats (15,16). Abortion causes are often unknown because of the complexity of the underlying mechanisms. However, it seems that infectious factors are the most common cause of mortality in the fetuses of sheep and goats. It is essential to recognize *N. caninum*'s contribution in causing abortion in sheep and goats (17). Multiple diagnostic strategies, including histopathological techniques, immunohistochemistry, serological tools, and PCR, have been developed by scientists to detect *N. caninum* infection in fetal abortions (18-21). Until recently,

neosporosis genetic identification has relied on various genes, including those for internal transcribed spacer (ITS) sequences, *18S rRNA*, and the Nc-5 genes. Meanwhile, due to its repetition in the *N. caninum* sequence, the *Nc-5* gene has been shown to have high sensitivity and specificity for identifying neosporosis (22).

The primary reason for conducting this research was to examine the feasibility of employing PCR with primers exclusive for the *Nc-5* gene for the diagnosis and genetic identification of neosporosis in sheep in Al-Qadisiyah Province, Iraq.

Materials and methods

Ethical approval

The current study was designed and conducted under the permission provided by the Ethical Approval Committee at the College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq, and according to national and international criteria for the animal care and use.

Samples

The investigation included 200 asymptomatic sheep (2-10 years old) samples (20 heart and 80 brain tissues and 100 blood) collected from different districts of Al-Qadisiyah Province, Iraq, from June 2022 to January 2023. Whole sheep heads and whole hearts were collected and placed in ice-cooled containers for the organs. The collected samples were transported immediately to the Laboratory of Parasitology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq, where the Lab work was performed directly.

DNA extraction

The DNA materials were extracted using the AddBio extraction kit (Korea) and following its protocol steps. The DNA obtained from the extraction process was evaluated for quality and quantity, utilizing a NanoDrop. The target DNA was placed in a -20ºC-storage.

Nc-5 gene PCR

After extracting the DNA, the samples were subjected to PCR. *Nc-5* gene-based primers (F: CAGTCAACCTACGTCTTC and R: GTGCGTCCAATCCTGTAA), design obtained from Li *et al.* (23), were used in the PCR reaction solution. The PCR solution (20µl of total volume) included (10, 1.5 (0.5pmol/each), 5, and 2µl) of master mix for each of F or R primer, PCR water, and DNA template, respectively. The steps of the thermocycler were under the following conditions: 95ºC-5mins, 95ºC-30s, 53ºC-35s, 72ºC-40s, and 72ºC-5mins of initial denaturation (one cycle), (denaturation, annealing, and extension) at 38 cycles, and final extension (one cycle). The PCR products of the target *Nc-5* gene were 1.5%-agarose-gel-run for an electrophoresis process at 100 volts and 80A for 60 mins. Then, the gel with the PCR product bands was evaluated by employing a UV imager.

Nc-5 gene-specific region sequencing

The PCR-purified products were *Nc-5* gene sequenced at the Macrogen company in Korea, recruiting a Sanger sequencing service. After that, the sequences were processed using NCBI-related websites and Mega X software to build a phylogenetic tree (24).

Results

Molecular findings

The finding of the PCR revealed the genetic identification of 7 (3.5%) isolates of *N. caninum* in the samples of the examined sheep (Figure 1). These positive samples were from the brain samples, with no detection from the heart samples. For the sequencing, seven PCR products were detected as *N. caninum* with nucleotide close similarity to isolates from some regions, such as New Zealand and Switzerland (Table 1 and Figure 2).

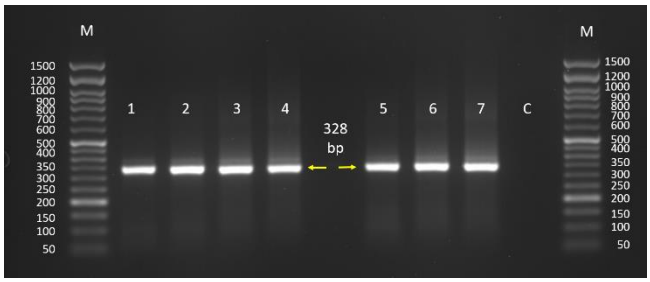

Figure 1: Image of 1.5%-agarose gel for the *Nc-5* gene-dependent PCR of *Neospora caninum* from sheep brain, heart, and blood samples. M: (50-1500bp) scaled ladder and lanes (1 to 7): positive PCR *Nc-5* products.

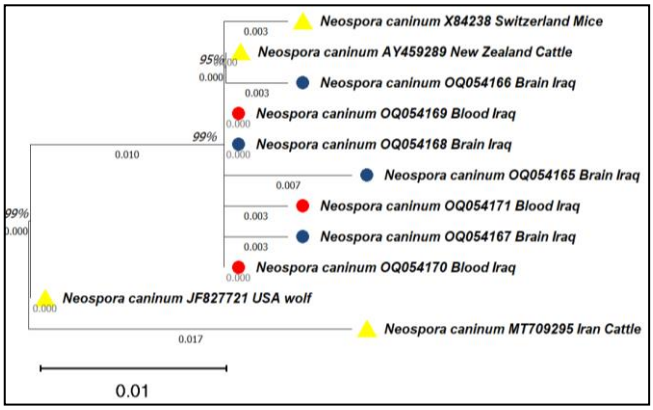

Figure 2: Phylogenetic tree for the *Nc-5* gene-dependent sequencing of *Neospora caninum* from sheep brain, heart, and blood samples.

Table 1: NCBI-BLAST homology sequence identity in local *Neospora caninum* in sheep of *Nc-5* gene

Accession number (current)	Accession number (world)	Country	Identity (%)	Host
OQ054165	X84238	Switzerland	99.32	mice
OQ054166	AY459289	New Zealand	100	Cattle
OQ054167	LN714488	UK	98.29	Undetermined
OQ054168	JF827721	USA	98.63	Wolf
OQ054169	KF649847	USA	97.95	wolf
OQ054170	MT709295	Iran	96.9	Cattle
OQ054171	KP715560	Italy	97.67	Deer

Discussion

There is an insufficient amount of data detailing the genetic-based detection of silent neosporosis in sheep despite reports of a correlation between ovine abortion and neosporosis (25-29). The current study found that the protozoan caused infection in 7 (3.5%) sheep. This data agrees with Arbabi *et al.* (30), who reported that the overall infection rate in their sheep samples (hearts and brains) was 3.9% in 13 out of 330 sheep. They found that the parasite was detected in 12 (6.7%) hearts and one (0.7%) brain. Previous research has shown that between 1.1 and 8.3% of sheep in western Iran test positive for antibodies to *N. caninum* (31,32). The DNA of *N. caninum* was also found in 8.5% and 0.9% of aborted fetuses in Iran (33,34).

Among 181 goat youngsters studied in Romania, the DNA of *N. caninum* was found only in 2 (1.1%) of the diaphragm tissues (35). In most investigations, aborted or spontaneously infected sheep brain samples were positive for *N. caninum* (33,34). For instance, in Brazil, Silva *et al.* (36) found the DNA of *N. caninum* in 2 per every 102 (1.9%) sacrificed goats. All heart and tongue specimens were negative for the parasite. However, both test results were extracted from brain tissues. In Brazil, Santos *et al.* (37) found the DNA of *N. caninum* in 5% of brain tissues from beef cattle but in 0% of heart tissues. Some research found that the majority of samples tested 6.7% were found in the heart specimens instead of the brain extracts 0.7% (37).

In this work, we employed the *Nc-5* gene to identify *N. caninum* and conduct a phylogenetic analysis. This gene is described as a DNA region with high sensitivity and specificity for identifying neosporosis since it is a repeated DNA fragment of *N. caninum* (38). Yamage *et al.* (39) examined the sensitivity and specificity of several primers for diagnosing *N. caninum*. To detect neosporosis in mice infected experimentally, they examined the sensitivity and specificity of Np1, Np3, Np5, Np7, and Np21 forward primers and Np2, Np4, Np6, and Np8 reverse primers generated from the Nc-5 genes (39). The Np21-Np6, Np7-Np6, and Np2l-Np4 primer sets were the only ones out of the 19 tested that could pinpoint a minimum of 10pg DNA with distinct separate bands (39). Similar apicomplexan pathogens, such as *Toxoplasma gondii* and Sarcocystis species, may be distinguished from *N. caninum* by its unique Nc-5 gene. This led to the Nc-5 gene being utilized as a susceptible and specific genetic material for identifying neosporosis. Because of its high sensitivity and specificity, the Nc-5 gene was chosen for this study neosporosis identification (30).

For the sequencing, seven PCR products were detected as *N. caninum* with nucleotide close similarity to isolates from some regions, such as New Zealand and Switzerland. These similarities could be because *N. caninum* can infect different animal species, which allows the protozoan to disseminate quickly to any country via any tool, such as importing animals (40-47).

Conclusion

The findings demonstrated by the herein-study that sheep can be a host for *Neospora caninum*, in which similar isolates are isolated from different countries; however, all these global isolates were reported only from species other than from sheep; the current study target animal.

Acknowledgments

The authors would like to thank the College of Veterinary Medicine, University of Al-Qadisiyah.

Conflict of Interest

There is no conflict of interest for the current work.

References

1. Reichel MP, Ayanegui-Alcérreca MA, Gondim LF, Ellis JT. What is the global economic impact of Neospora caninum in cattle-the billion dollar question. Int J Parasitol. 2013;43(2):133-42. [available at]
2. Villa L, Gazzonis AL, Fumagalli E, Zanzani SA, Manfredi MT. The utility of serological analysis for *Neospora caninum* infection in dairy cattle farms management: Serological investigation and evaluation of the effects on reproductive and productive performances in two study herds in northern Italy. Anim. 2022;12(6):786-96. DOI: 10.3390/ani12060786
3. González-Warleta M, Castro-Hermida JA, Calvo C, Pérez V, Gutiérrez-Expósito D, Regidor-Cerrillo J, Ortega-Mora LM, Mezo M. Endogenous transplacental transmission of *Neospora caninum* during

successive pregnancies across three generations of naturally infected sheep. Vet Res. 2018;49(1):106-17. DOI: 10.1186/s13567-018-0601-3
4. Wilson DJ, Orsel K, Waddington J, Rajeev M, Sweeny AR, Joseph T, Grigg ME, Raverty SA. *Neospora caninum* is the leading cause of bovine fetal loss in British Columbia, Canada. Vet Parasitol. 2016;218(3):46-51. DOI: 10.1016/j.vetpar.2016.01.006
5. Mazuz ML, Fish L, Reznikov D, Wolkomirsky R, Leibovitz B, Savitzky I, Golenser J, Shkap V. Neosporosis in naturally infected pregnant dairy cattle. Vet Parasitol. 2014;205(1-2):85-91. DOI: 10.1016/j.vetpar.2014.06.009
6. Iovu A, Györke A, Mircean V, Gavrea R, Cozma V. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy goats from Romania. Vet Parasitol. 2012;186(3-4):470-4. DOI: 10.1016/j.vetpar.2011.11.062
7. Gharekhani J, Esmaeilnejad B, Rezaei H, Yakhchali M, Heidari H, Azhari M. Prevalence of anti-*Neospora caninum* antibodies in Iranian goats. Ann Parasitol. 2016;62(2):111-4. [available at]
8. Gazzonis A, Villa L, Manfredi M, Zanzani S. Spatial analysis of infections by *Toxoplasma gondii* and *Neospora caninum* (Protozoa: Apicomplexa) in small ruminants in northern Italy. Anim. 2019;9(11):916-29. DOI: 10.3390/ani9110916
9. Nasir A, Ashraf M, Khan MS, Javeed A, Yaqub T, Avais M, Reichel MP. Prevalence of *Neospora caninum* antibodies in sheep and goats in Pakistan. J Parasitol. 2012;98(1):213-5. DOI: 10.1645/ge-2863.1
10. Basso W, Holenweger F, Schares G, Müller N, Campero LM, Ardüser F, Moore-Jones G, Frey CF, Zanolari P. *Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland: Seroprevalence and occurrence in aborted fetuses. Food Waterborne Parasitol. 2022;28(9):e00176-88. DOI: 10.1016/j.fawpar.2022.e00176
11. Anastasia D, Elias P, Nikolaos P, Charilaos K, Nektarios G. *Toxoplasma gondii* and *Neospora caninum* seroprevalence in dairy sheep and goats mixed stock farming. Vet Parasitol. 2013;198(3-4):387-90. DOI: 10.1016/j.vetpar.2013.09.017
12. Rodrigues AA, Reis SS, da Silva Moraes E, do Nascimento Souza Filho JG, dos Santos Reis MH, Martins TA, Bernardes JC, Nino BD, Garcia JL, Nascimento TV, da Cunha IA. Seroprevalence and risk factors for *Neospora caninum* and *Toxoplasma gondii* in goats of Maranhão state, Brazil. Vet Parasitol Reg Stud Rep. 2021;26(12):100634-7. DOI: 10.1016/j.vprsr.2021.100634
13. Romanelli PR, Caldart ET, Martins FD, Martins CM, de Matos AM, Pinto-Ferreira F, Mareze M, Mitsuka-Breganó R, Freire RL, Navarro IT. Seroprevalence and associated risk factors of ovine neosporosis worldwide: A systematic review and meta-analysis. Semin Cienc Agrar. 2021;42(3):2111-25. DOI: 10.5433/1679-0359.2021v42n3Supl1p2111
14. Hoste H, Sotiraki S, Landau SY, Jackson F, Beveridge I. Goat-nematode interactions: Think differently. Trends Parasitol. 2010;26(8):376-81. DOI: 10.1016/j.pt.2010.04.007
15. Benavides J, González-Warleta M, Arteche-Villasol N, Pérez V, Mezo M, Gutiérrez-Expósito D. Ovine neosporosis: The current global situation. Anim. 2022;12(16):2074-93. DOI: 10.3390/ani12162074
16. Rodrigues AA, Reis SS, de Sousa ML, da Silva Moraes E, Garcia JL, Nascimento TV, da Cunha IA. A systematic literature review and meta-analysis of risk factors for *Neospora caninum* seroprevalence in goats. Prev Vet Med. 2020;185(12):105176-85. DOI: 10.1016/j.prevetmed.2020.105176
17. Nayeri T, Sarvi S, Moosazadeh M, Daryani A. The global prevalence of *Neospora caninum* infection in sheep and goats that had an abortion and aborted fetuses: A systematic review and meta-analysis. Front Vet Sci. 2022;9(4):870904-15. DOI: 10.3389/fvets.2022.870904
18. Uzêda RS, Schares G, Ortega-Mora LM, Madruga CR, Aguado-Martinez A, Corbellini LG, Driemeier D, Gondim LF. Combination of monoclonal antibodies improves immunohistochemical diagnosis of *Neospora caninum*. Vet Parasitol. 2013;197(3-4):477-86. DOI: 10.1016/j.vetpar.2013.07.008
19. Tian AL, Elsheikha HM, Zhou DH, Wu YD, Chen MX, Wang M, Chen D, Zhang XC, Zhu XQ. A novel recombinase polymerase amplification (RPA) assay for the rapid isothermal detection of Neospora caninum in aborted bovine fetuses. Vet Parasitol. 2018;258(7):24-9. DOI: 10.1016/j.vetpar.2018.06.004
20. Pescador CA, Corbellini LG, Oliveira EC, Raymundo DL, Driemeier D. Histopathological and immunohistochemical aspects of *Neospora caninum* diagnosis in bovine aborted fetuses. Vet Parasitol. 2007;150(1-2):159-63. DOI: 10.1016/j.vetpar.2007.08.028
21. Della Rosa P, Fiorentino MA, Morrell EL, Scioli MV, Paolicchi FA, Moore DP, Cantón GJ, Hecker YP. *Neospora caninum* and *Toxoplasma gondii* are causes of reproductive losses in commercial sheep flocks from Argentina. Curr Res Parasitol Vector-borne Dis. 2021;1(11):100057-63. DOI: 10.1016/j.crpvbd.2021.100057
22. Dubey JP, Jenkins MC, Ferreira LR, Choudhary S, Verma SK, Kwok OC, Fetterer R, Butler E, Carstensen M. Isolation of viable *Neospora caninum* from brains of wild gray wolves (*Canis lupus*). Vet Parasitol. 2014;201(1-2):150-3. DOI: 10.1016/j.vetpar.2013.12.032
23. Li J, He P, Yu Y, Du L, Gong P, Zhang G, Zhang X. Detection of *Neospora caninum*-DNA in feces collected from dogs in Shenyang (China) and ITS1 phylogenetic analysis. Vet Parasitol. 2014;205(1-2):361-4. DOI: 10.1016/j.vetpar.2014.06.036
24. Stecher G, Tamura K, Kumar S. Molecular evolutionary genetics analysis (MEGA) for macOS. Mol Biol Evol. 2020;37(4):1237-9. DOI: 10.1093/molbev/msz312
25. Magaña A, Sánchez F, Villa K, Rivera L, Morales E. Systemic neosporosis in a dog treated for immune-mediated thrombocytopenia and hemolytic anemia. Vet Clin Pathol. 2015;44(4):592-6. DOI: 10.1111/vcp.12287
26. Mazuz ML, Shkap V, Wollkomirsky R, Leibovich B, Savitsky I, Fleiderovitz L, Noam S, Elena B, Molad T, Golenser J. *Neospora caninum*: Chronic and congenital infection in consecutive pregnancies of mice. Vet Parasitol. 2016;219(3):66-70. DOI: 10.1016/j.vetpar.2016.01.013
27. Pena HF, Soares RM, Ragozo AM, Monteiro RM, Yai LE, Nishi SM, Gennari SM. Isolation and molecular detection of *Neospora caninum* from naturally infected sheep from Brazil. Vet Parasitol. 2007;147(1-2):61-6. DOI: 10.1016/j.vetpar.2007.03.002
28. Moreno B, Collantes-Fernández E, Villa A, Navarro A, Regidor-Cerrillo J, Ortega-Mora LM. Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. Vet Parasitol. 2012;187(1-2):312-8. DOI: 10.1016/j.vetpar.2011.12.034
29. Howe L, Collett MG, Pattison RS, Marshall J, West DM, Pomroy WE. Potential involvement of *Neospora caninum* in naturally occurring ovine abortions in New Zealand. Vet Parasitol. 2012;185(2-4):64-71. DOI: 10.1016/j.vetpar.2011.10.033
30. Arbabi M, Abdoli A, Dalimi A, Pirestani M. Identification of latent neosporosis in sheep in Tehran, Iran by polymerase chain reaction using primers specific for the Nc-5 gene. Onderstepoort J Vet Res. 2016;83(1):1058-64. DOI: 10.4102/ojvr.v83i1.1058
31. Ezatpour B, Alirezaei M, Hassanvand A, Zibaei M, Azadpour M, Ebrahimzadeh F. The first report of *Neospora caninum* prevalence in aborted and healthy sheep from the west of Iran. Comp Clin Path. 2015;24(1):19-22. DOI: 0.1007/s00580-013-1846-x
32. Gharekhani J, Heidari H. Serology-based comprehensive study of Neospora infection in domestic animals in Hamedan province, Iran. J Adv Vet Anim Res. 2014;1(3):119-24. DOI: 10.5455/javar.2014.a23
33. Sasani F, Javanbakht J, Seifori P, Fathi S, Hassan MA. *Neospora caninum* as causative agent of ovine encephalitis in Iran. Pathol Discov. 2013;1(1):9. DOI: 10.7243/2052-7896-1-5
34. Asadpour R, Jafari-Joozani R, Salehi N. Detection of *Neospora caninum* in ovine abortion in Iran. J Parasit Dis. 2013;37(1):105-9. DOI: 10.1007/s12639-012-0141-0
35. Şuteu O, Paştiu A, Györke A, Avram E, Cozma V. Molecular detection of *Neospora caninum* in slaughtered goat kids from Romania. Sci Parasitol. 2013;14(1):43-6. [available at]
36. Silva MS, Uzêda RS, Costa KS, Santos SL, Macedo AC, Abe-Sandes K, Gondim LF. Detection of *Hammondia heydorni* and related coccidia (*Neospora caninum* and *Toxoplasma gondii*) in goats slaughtered in Bahia, Brazil. Vet Parasitol. 2009;162(1-2):156-9. DOI: 10.1016/j.vetpar.2009.02.007

دراسة جزيئية ونشئية معتمدة على الجين ن س-خامس في النيوسبورا الكلبية في الأغنام

أزهار جفات كروان ومنصور جدعان الخالد

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة القادسية، الديوانية، العراق

الخلاصة

أجريت الدراسة الحالية للتعرف على تواجد طفيلي النيوسبورا الكلبية في الأغنام باستخدام التقنيات الجزيئية. اشتمل الدراسة على ٢٠٠ عينة غنم (٢٠ عينة قلب و٨٠ عينة دماغ و ١٠٠ عينة دم) جمعت من مناطق مختلفة من محافظة القادسية، العراق. تم إخضاع العينات، بعد استخلاص الحمض النووي الريبوز منقوص الأوكسجين، لتقنيات تشخيصية جزيئية؛ تفاعل أنزيم البلمرة المتسلسل ودراسة تعاقب القواعد النيتروجينية الجزئي المصحوب بتقدير حالة التطور في الكائنات الحية النيوسبورا الكلبية. اعتمدت كلتا الطريقتين على اكتشاف تواجد جين النيوسبورا الكلبية الخامس، وهي قطعة متكررة في الحمض النووي الكائن الحي. أظهرت نتائج اختبار تفاعل انزيم البلمرة المتعدد التعرف الوراثي على ٧ (٣,٥%) عزلات من النيوسبورا الكلبية في عينات الأغنام التي تم فحصها. بالنسبة لدراسة تعاقب القواعد النيتروجينية الجزئي، تم تحديد سبع منتجات باستخدام تفاعل أنزيم البلمرة المتسلسل على أنها النيوسبورا الكلبية مع تشابه قريب في القواعد النيتروجينية مع عزلات من بعض مناطق العالم، مثل نيوزيلندا وسويسرا. أظهرت النتائج من خلال الدراسة الواردة هنا أن الأغنام يمكن أن تكون مضيف وسطي للنيوسبورا الكلبية؛ ومع ذلك، تم تحديد كل هذه العزلات العالمية فقط من أنواع أخرى غير الأغنام؛ الحيوان المستهدف في الدراسة الحالية.

37. Santos SL, de Souza Costa K, Gondim LQ, da Silva MS, Uzêda RS, Abe-Sandes K, Gondim LF. Investigation of *Neospora caninum*, *Hammondia sp.*, and *Toxoplasma gondii* in tissues from slaughtered beef cattle in Bahia, Brazil. Parasitol Res. 2010;106(2):457-61. DOI: 10.1007/s00436-009-1686-4
38. Al-Qassab SE, Reichel MP, Ellis JT. On the biological and genetic diversity in *Neospora caninum*. Divers. 2010;2(3):411-38. DOI: 10.3390/d2030411
39. Yamage M, Flechtner O, Gottstein B. *Neospora caninum*: Specific oligonucleotide primers for the detection of brain "cyst" DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). J Parasitol. 1996;82(2):272-9. DOI: 10.2307/3284160
40. Almería S. *Neospora caninum* and Wildlife. ISRN Parasitol. 2013;2013(6):1-23. DOI: 10.5402/2013/947347
41. Nooraldin MY, Jaafar SA, Salih AI. Seroprevalence of *Neospora caninum* infections in cattle in Kirkuk province. Iraqi J Vet Sci. 2021;35(2): 331-334. DOI: 10.33899/ijvs.2020.126832.1394
42. Al-Musawi AM, Awad AH, Alkhaled MJ. Molecular analysis of Cryptosporidium species in domestic goat in central Iraq. Iraqi J Vet Sci. 2022;36(4):1041-1045. DOI: 10.33899/ijvs.2022.132974.2155
43. Kadhum NK, Karim SM, Mansour KA, Al-Fatlawi MA. Indicative parameters for liver fascioliasis at pre-clinical and clinical phases in cows from Al-Diwaniyah city, Iraq. Iraqi J Vet Sci. 2022;36(3):653-657. DOI: 10.33899/ijvs.2022.132266.2076
44. Ali IF, Jihad TW. Perturbation of liver function markers and serum electrolytes associated with Echinococcus granulosus infection in sheep. Iraqi J Vet Sci. 2022;36(1):65-69. DOI: 10.33899/ijvs.2021.128926.1624
45. Ismail HK, AL-Saleem IA, Jasim AY. Experimental study on the effect of toxin fractions isolated from hydatid cyst fluid of sheep on the cardiac muscles of mice. Iraqi J Vet Sci. 2021;35(3):523-528. DOI: 10.33899/ijvs.2020.127124.1463
46. Yahya MQ, Mohi-Aldeen ZK. Secondary bacterial infection of hydatid cysts infected livestock animals (In vitro study). Iraqi J Vet Sci. 2022;36(3):761-768. DOI: 10.33899/ijvs.2022.131873.2016
47. Jwher DM, Jarjees MT, Alshater AM. A study of the gastrointestinal parasites in awassi sheep and surrounding environment. Iraqi J Vet Sci. 2021;35(3): 561-567. DOI: 10.33899/ijvs.2020.127174.1478