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Molecular detection of *Neospora caninum* in domestic and stray dogs in Babylon province, Iraq.

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Abstract Neospora caninum is one of the most important intracellular parasites, which infect dogs as intermediate and final host, as well as domestic animals as intermediate host, to result in severe economic losses. and causes neurological disorders and venereal problems in dogs. This study was conducted to detect N.caninum in domestic and a stray dogs and also to determine the effect of sex, age, and month on the incidence rate in Babylon Province.

Whole of 100 blood specimen were collected during the period from the beginning of July to the end of December 2024. dogs Neosporosis was detected in 9%(9/100) using conventional PCR. A high incidence rate was recorded in stray dogs11.1% (6/54) compared to domestic dogs which recorded an incidence rate of 6.5% (3/46) with no a great difference at P≥0.05. The prevalence rate in males was 9.6% (5/52) while in females 8.3% (4/48) without a great difference at P≥0.05. The highest infection rate was observed in adult domestic dogs7.6% (2/26) while the lowest rate was in puppies 5% (1/20) without great difference at P≥0.05. The prevalence rate changed during the different months studied where the highest and lowest rates (17.6% and 4.5%) were recorded in September and December respectively without great difference at P≥0.05. The present study used Molecular diagnostic examination methods particularly (conventional PCR) to identify the critical role of astray and domestic dogs in dissemination of N.caninum infection among animals in Babylon Province, Iraq.

Keywords: Neosporosis, Dogs, Babylon, PCR, Blood.

Introduction The apicomplexan protozoan *Neospora caninum* is widely distributed geographically and causes significant financial stress on farmers and the livestock industry (1).Dogs in Norway were first identified with this infection in 1984 (2).The parasite shares a large number of morphologic and biological characteristics with its close relative *Toxoplasma gondii* (3).It is a heteroxenous parasite that requiring multiple hosts to complete its life cycle (4). *N.caninum* has two reproductive forms in life cycle asexual reproduction which occurs in intermediate hosts such as cattle and sexual reproduction which only occurs in definitive hosts such as canids (5).

Vertical transmission happens either during the last stage of pregnancy or through milk after birth (6) while horizontal transmission involves consumption of infected tissues from intermediate hosts harboring tissue cysts (7).The parasite can cause heavy disease in all ages of dogs (8). Infected dogs exhibit neuromuscular symptoms including encephalomyelitis and myositis which can cause paralysis and premature puppy death and depending on the location the parasite in the central nervous system adult dogs may exhibit a variety of neurological symptoms including rigid hyperextension and paralysis of the pelvic limbs (9).

One of the most important instruments for diagnosing *N.caninum* infection is the polymerase chain reaction (PCR) it can identify *N.caninum* DNA (10). The PCR analysis is a highly sensitive method that can test tissues in addition to blood and other fluids(11). The deficiency in the information around the molecular detection of *N.caninum* in dog blood samples and the effect of risk agents on the infection rate in Babylon province therefore this study was designed.

Material and Methods

Ethical approval

The study was conducted in compliance with ethical guidelines for animal use and care, as approved by the University of Al-Qadisiyah College of Veterinary

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Medicine. On November 17, 2024, the this study was approved under Approval Number. 4953.

Collection of samples

From July to December 2024, 100blood samples were drawn from the jugular or cephalic veins of randomly selected domestic and stray dogs from various locations in Babylon Province. This exercise was performed using a sterile syringe and a disposable glove tubes containing EDTA as an anticoagulant samples of blood kept cool throughout transportation to the Parasitological Lab. in the College of Veterinary Medicine University of Al-Qadisiyah and use them for PCR testing. All specimen used for PCR testing were stored and preserved at -20°C**.**

DNA isolation and conventional PCR.

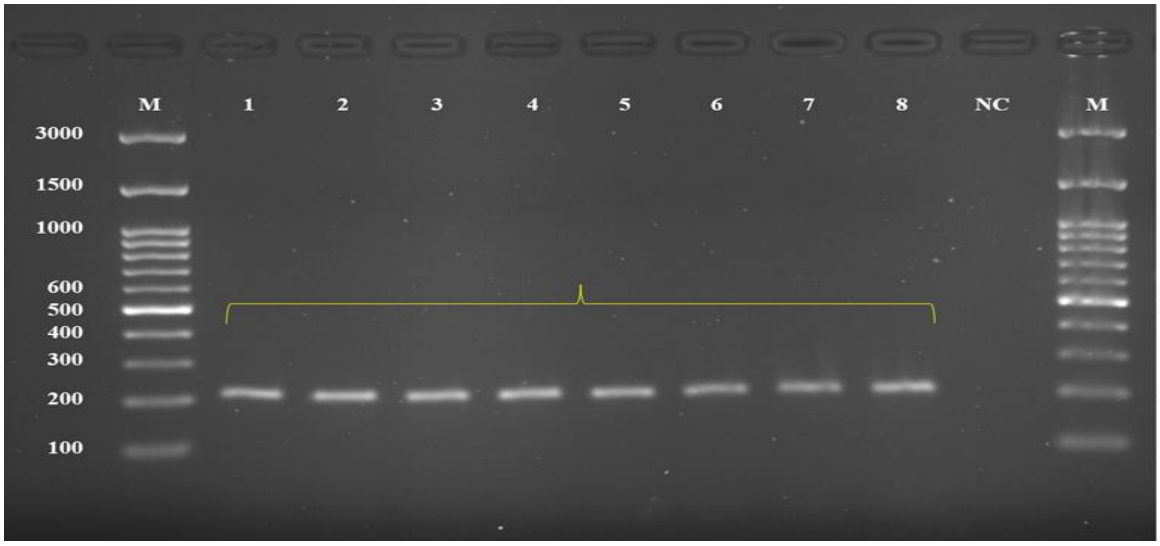
The Genomic DNA Extraction kit Blood (Addbio, Korea) was used to extract genomic DNA from blood samples of 100 stray and domestic dogs. This was performed as per the protocol described in the manufacturer's instructions. The extracted DNA was stored at −20°C, Primers were designed in this study targeting a specific partial region within the putative peptidase family M48 domain-containing protein-encoded gene to identify *N. caninum*.The primers. Table (1).

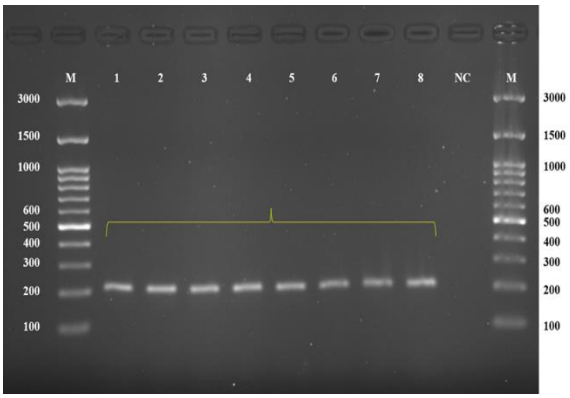
Table (1): Primers for Conventional PCR used in this study.

Primer	Sequence 5'------ 3'	Start	End	Accession No.	Product size
Forward	AGCAACGCGT ACAACAAAGA	1356 50	1356 69	LN71447 6	204 bp
Reverse	GGAGATACA CTCGCCGAT CA	1358 34	1358 53		

Statistical analysis

Data were collected, tabulated, and statistically analyzed using a personal computer with the "Statistical Package for the Social Sciences (SPSS) version 26 program (IBM Corporation, Armonk, NY, USA). The chi-square test was used to evaluate qualitative data. A value of $P < 0.05$ was considered statistically significant (12).





Results

Molecular Detection of dogs Neosporosis in blood samples by conventional PCR.

Depending on PCR analysis that focused on a partial region within the putative peptidase family M48 domain-containing protein-encoded gene, only 9dogs out of 100 blood samples were infected. Figure (1).

Figure (1): Agarose gel electrophoresis image (1.5% agarose)shows the ampliconof *Neospora caninum* targeting the partial region (204 bp) of the putative peptidase family M48 domain-containing protein-encoded gene (1-8) detected from blood sample. NC is a non-template control in which H2O was added instead of template DNA. M is a molecular marker from Genedirex, Korea.

Table (2) : The NCBI-BLAST Homology Sequence identity (%) in local Neospora caninum targeting putative peptidase family M48 domain-containing protein within gene. These sequences were deposited

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in gene bank under the following accession numbers (PQ817119, PQ817120, PQ817121, PQ817122, PQ817123, PQ817124, PQ817125, PQ817126, PQ817127, and PQ817128) and these were being compared with other global sequences

Sequence number	**Obtained Accession number**	**Source**	**Identical to**	**Genbank Accession number**	**Country**	**Identity (%)**
1	PQ817119	Blood	Neospora caninum	LN714476	UK	99
2	PQ817120	Blood	Neospora caninum	LN714476	UK	99.5
3	PQ817121	Blood	Neospora caninum	LN714476	UK	97.01
4	PQ817122	Blood	Neospora caninum	LN714476	UK	99
5	PQ817123	Blood	Neospora caninum	LN714476	UK	97.51

Infection rate of *Neospora caninum* as per type of dogs .

In stray dogs 11.1% (6/54),shows incidence rate more than in domestic dogs, 6.5% (3/46),no significant variance at P≥0.05.Table (3).

Table (3): whole incidence rate and distribution of Neosporosis according to canine type .

Dogs	No. of examined samp	Positive	%
Stray	54	6	11.1
Domestic	46	3	6.5
Total	100	9	9
Chi-Square (χ		0.639	
P value		0.424(NS)	

NS: No significant variance at P≥0.05.

Infection rate depending on sex.

Male dogs exhibited a higher rate of infection at 9.6% (5/52), whereas female dogs had a lower infection rate of 8.3% (4/48).Table (4) reflects that this variance has no statistical significance at P≥0.05.

Table (4) :Infection rate of canine neosporosis as per sex.

Sex	No. of examined sam	Positive	Percentage (%)
Males	52	5	9.6
Females	48	4	8.3
Total	100	9	9
Chi-Square ($\chi 2$)		0.050	
P value		0.823 (NS)	

NS: No significant variance at P≥0.05.

Infection rate in household dogs as per age.

Among domestic dogs, the infection rate was lowest in the puppies (young age group) at 5% (1/20), whereas the infection rate was highest in the adult age group at 7.6% (2/26) without significant statistical variance at P≥0.05.Table (5).

Table (5): Infection rate of Neosporosis in household dogs depending on age.

Age	No. of examined samp	Positive	(%)
puppies	20	1	5
Adult	26	2	7.6
Total	46	3	6.5
Chi-Square		0.134	
P value		0.714 (NS)	

NS: No significant variance at P≥0.05.

Infection rate in months.

Table (6) shows that the incidence of *N. caninum* was highest in September (17.6%), then October (14.2%), whereas there was a lower incidence in November (5%), followed by December (4.5%) without significant statistical variance at P≥0.05.

Table (6): Infection rate of canine Neosporosis as recognized per month.

Months	No. of examined sample	Positive	Percentage (%)
July	15	1	6.6
August	12	1	8.3
September	17	3	17.6
October	14	2	14.2
November	20	1	5
December	22	1	4.5
Chi-Square (χ		3.06	
P value		0.691(NS)	

NS: No significant variance at P≥0.05.

Discussion

Dogs play a significant role in sustaining the infection and facilitating a storm of abortion because of the horizontal spread of *N.caninum* to domestic animals (13). PCR finding specific DNA in limited specimen is one of the most accurate extensively used molecular approaches to finding the global prevalence of *N. caninum* infection in animals (14). The total infection rate of *N. caninum* in dog blood samples in Babylon province, Iraq, was 9% (9/100) this result is more than that observed in the study performed by (15) recorded the infection rate of dog blood samples by PCR in several provinces in Thailand. It was recorded in Lopburi Province as 0.7% while in Nakhon Pathom Province it was 7.4% in Sa Kaeo Province it was 3.8% and in Ratchaburi Province it was 5.8%. While the result lower than that recorded in Al-Nassiriya Province, Iraq (16) recorded a rate of 18.4% in dairy and beef cattle by molecular diagnosis. These differences may be associated with several aspects, such as the number of dogs in the location;climate humidity induces sporulation and enhances the survival of *N. caninum* oocysts in the ecosystem (17).

In this study, we recorded a higher infection rate in a stray dogs than in domestic dogs. These results agree with those of(18), (19). It has been shown that dogs living in the countryside have asignificantly greater risk of *N.caninum* infection than dogs living in large cities since they have direct access to parasite cysts present in placentas, fetuses, or tissues from infected livestock (20).

The results of this study showed a higher infection rate in males than in females. Those results agree with (21) in Algiers, which reported a higher infection rate in male dogs, and (22). Due to the different diagnostic techniques used and environmental and climatic conditions, the efficacy of the immune system's reaction (23).

The result recorded the infection rates of *N. caninum* among different age groups, lowest in the puppies age group, while the infection was highest in the adult age group. This result agrees with (15), who reported the incidence was more prevalent in adult dogs. (24) reported *N. caninum* increases with the age of dogs. Another study in northeastern Italy reported dogs of any age could be infected, with an increased prevalence reported in older dogs compared to younger ones (25). In the Algiers District, a higher infection rate was reported in adult dogs (22). The high infection in older animals is due to their chance of exposure to infection over time (26).

We conducted a study in Babylon province, Iraq, to detect the parasite *N. caninum* in dog blood samples using molecular diagnosis. The results were according to the month's highest infection rate in September and October, while the lowest infection rate was recorded in November and December. Other researchers have also conducted studies to detect *N. caninum* in dogs and other animals as intermediate hosts, according to the months. (22) conducted a study in the Algiers District in dogs according to months: winter 19%, spring 24.4%, summer 45.1%, and autumn 18.3% . (27) conducted a study in Babylon Province,Iraq in in blood samples from aborted cows by PCR according to months: autumn 18.3%,winter 19%.Higher temperatures may favor a faster sporulation of oocysts in the environment surrounding the dogs (28).

Conclusion

This result showed that *N. caninum* is present in the Babylon province due to its high incidence in dogs (stray, male, adult) as well as in specific months, which confirms the requirement for routine monitoring of this parasite to avert economic losses across the dissemination of its occurrence to farm animals.

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Conflict of interest

The authors declare no conflict of interest

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