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Molecular detection of *Neospora caninum* in the placentas and umbilical cord blood of women who experienced abortions

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RESEARCH ARTICLE

Molecular detection of *Neospora caninum* in the placentas and umbilical cord blood of women who experienced abortions

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ABSTRACT

The relationship between Neosporosis infection in humans and its potential role in causing abortion remains uncertain. This is a molecular analytical cross-sectional study *of Neospora caninum* in the placentas and umbilical cord blood from aborted women. The results will be compared with molecular examinations of the placentas from several aborted livestock. 157 samples of placentas from aborted cows (58), sheep (51), and goats (48) were collected. In addition, 115 aborted fetal samples of cows (42), sheep (40) and a goat (33) were collected. Also, from aborted women, 45 samples were collected from the placentas and 45 samples of umbilical cord blood in the governorates of Muthanna and Qadisiyah in the Middle Euphrates region in Iraq. The overall infection rate was 5.45%, with 11 out of 202 samples taken from animals and women testing positive for infection. The highest infection rates were observed in older animals. No traces of *N. caninum* were found in the DNA samples extracted from the women's placentas. However, the DNA of the parasite was present in the blood of the umbilical cord in 4.4% of human embryos, as it was detected in 2 out of 45 samples. The presence of *N. caninum* in the umbilical cord blood indicates that the mother has acquired an infection. However, the absence in the placenta may be due to the parasite subspecies, which might not be able to penetrate the placental walls, or it could be that the human immune system has defenses that prevent the formation of the bradyzoite phase.

Keywords: Abortion, Nc-5 gene, Neospora caninum, Placenta, Umbilical cord blood

Introduction

N. caninum is a protozoan that causes neosporosis, a worldwide disease that negatively affects reproductive efficiency in cattle. This leads to significant economic losses in the livestock industry, as the disease can cause abortions and often results in the birth of congenitally infected animals.[1] Additionally, dogs infected with N. caninum can display various neuromuscular symptoms.[2]

N. caninum is an obligatory endocellular life organism, not zoonotic, with a heteroxen life cycle, where two types of hosts intervene, the final hosts (carnivores) in which the sexual phase is completed, and the intermediaries (herbivores) in which the asexual phase is carried out.[3] Three infective states: tachyzoites, tissue cysts, and oocysts. Tachyzoites and tissue cysts are intracellular stages of the infection in hosts. They can be found in different cell types, and each host cell can contain several tachyzoites, which are housed inside the parasitophorous vacuole in the cytoplasm.[4] Once inside the intermediate host, the parasite forms tissue cysts, which can remain dormant until reactivation during pregnancy. During this reactivation, the parasite migrates to the placenta, leading to abortion or congenital infection in the fetus.[5]

There are two transmission routes, the horizontal and the vertical routes. Horizontal transmission occurs when an animal is infected either by the intake of tachyzoites and/or tissue cysts or by consuming water and/or food contaminated with sporulated oocysts.[6]

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Vertical transmission (transplacental) occurs during pregnancy, when the fetus acquires the infection through its mother and is usually the most common and effective transmission route in cattle.[7]

The clinical signs before the infection by *N. Caninum* will be determined by the age of the animal, sex, immune state, number and infectious form, the individual genetic predisposition, the part of the central nervous system (CNS) affected, and, in the case of pregnant females, by the moment of gestation in which the infection occurs. The main clinical sign in cattle is abortion, although it can also be observed in exceptionally weak calves, with nervous signs,[1] and it can cause developmental effects in the offspring in cattle.[8] However, in most cases, in pregnant bovine females infected with *N. caninum,* the majority of bovine abortions caused by *N. Caninum* occur between 4 and 7 months of gestation.[9]

Materials and methods

Ethical approval

This study was prepared in accordance with the ethics approval protocol issued by the Ministry of Health and the Veterinary Department of the Ministry of Agriculture.

Samples and region

This study is an analytical cross-sectional study that included aborted livestock and women. From February 2024 to May 2025, 157 samples of placentas from aborted cows (58), sheep (51), and goats (48) were collected. In addition, 115 aborted fetal samples from cows (42), sheep (40) and a goat (33) were collected from veterinary clinics and private veterinary clinics. Also, from aborted women, 45 samples were collected from the placentas and 45 samples of umbilical cord blood from obstetrics in the governorates of Muthanna and Qadisiyah in the Middle Euphrates region (44.24 and 45.49 east) in Iraq.

With the assistance of a veterinarian or obstetrician, 2-3 grams of placenta and aborted embryos were collected and stored in sterile containers. Additionally, 5 millilitres of umbilical cord blood were collected in tubes containing EDTA K2. All samples were maintained at a temperature of -20 °C.

DNA extraction

Following the manufacturer's instructions from Pioneer in South Korea, we extracted DNA from tissues and blood samples. The quantity and quality of the extracted DNA were evaluated using the NanoDrop. The targeted DNA was stored at -20 °C.

Primer and gene

The Nc-5 gene was amplified using the method of Li et al. (2023)[10] and the primers (F: CAGTCAACCTACGTCTTC and R: GTGCGTCCAATCCTGTAA). In brief, the PCR reaction solution consisted of a total volume of 20 μl. It included 10 μl of master mix, 1.5 μl (0.5 pmol each) of forward (F) and reverse (R) primers, 5 μl of PCR water, and 2 μl of DNA template. The thermocycler steps were conducted under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 38 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 35 seconds, and extension at 72°C for 40 seconds. A final extension was performed at 72°C for 5 minutes.

The PCR products of the target Nc-5 gene were then analyzed by running them on a 1.5% agarose gel for electrophoresis at 100 volts and 80 A for 60 minutes. The gel, containing the bands of PCR products, was evaluated using a UV imager.

Gene sequence

The PCR-purified products of the Nc-5 gene were sequenced at Bioneer, Korea, using Sanger sequencing. The sequences were then processed with NCBI tools and Mega X software to create a phylogenetic tree.

Results

According to the results presented in Table 1, the overall infection rate was 5.45%, with 11 out of 202 samples taken from animals and women testing positive for infection. The highest infection rates were observed in older animals, with rates of 6.9%, 7.8%, and 6.25% found in placenta samples from cows, sheep, and goats, respectively. The younger animals did not show any signs of infection.

No traces of the parasite were found in the DNA samples extracted from the women's placentas. However, the DNA of the parasite was present in the blood of the umbilical cord in 4.4% of human embryos, as it was detected in 2 out of 45 samples Table 1.

The sequenced samples did not completely align with those in GenBank, with alignment percentages ranging from 99.17% to 98.47%, indicating the presence of unique Iraqi isolates Table 2. On the other hand, there is a gap in the evolutionary line of secondary species that infected humans and similar ones that infected livestock Fig. 1; Table 3.

Table 1. The prevalence of the parasite in molecular diagnostic samples of placenta, fetal and, umbilical cord blood, according to age.

Animals	Age (years)	Samples NO.	Infected(%)	Total	Embryo
Cows	< 2	13	0(0)	4/58 (6.9%)	5/42(11.9%)
	2-3	22	1(4.5)		
	> 3	23	3(13)		
Sheep	< 1	11	0(0)	4/51 (7.8%)	5/40(12.5%)
	1-2	14	2(14.2)		
	< 2	26	2(7.7)		
Goats	< 1	7	0(0)	3/48 (6.25%)	4/33(12.1%)
	1-2	18	1(11.1)		
	< 2	23	2(8.7)		
women	20-28	13	0(0)	0/45 (0%)	2/45(4.4%) (umbilical cord blood)
	29-37	15	0(0)		
	38-46	17	0(0)		
		202	11(5.45)		16/202(7.9%)

Table 2. Aligning the percentage range between the sequenced samples and similar samples deposited in Genebank.

Accession No.	Host	Accession No. (world)	Host	Identity	Country
KQ072161	Cows	X84238	Cow	99.17%	Switzerland
KQ072162		PP265560	Goat	99.17%	Iraq
KQ072163		JN106453	Cow	98.35%	China
KQ072164	Sheep	AY459289	Cow	98.33%	New zland
KQ072165		EU686398	Rabbite	97.5%	UK
KQ072166		MN969034	Domestic	98.3%	China
KQ072167	Goats	JF937545	-	99.17%	Australia
KQ072168		DQ132437	Fox	98.3%	USA
KQ072169	Humans	OL825740	Sheep	98.47%	Iran
KQ072170		OM484268	Sheep	98.47%	Iran

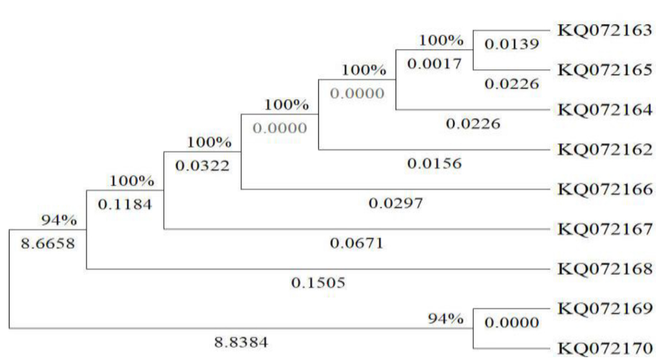

Fig. 1. The level of symmetry between the secondary species that affects humans and other animals.

Table 3. The evolutionary line gap between secondary species that infected humans and those that infected livestock.

Sequences	Host	KQ072162	KQ072163	KQ072164	KQ072165	KQ072166	KQ072167	KQ072168	KQ072169
KQ072162	Cow								
KQ072163		0.0315							
KQ072164	Sheep	0.0387	0.0387						
KQ072165		0.0405	0.0368	0.0423					
KQ072166		0.0459	0.0459	0.0532	0.0550				
KQ072167	Goats	0.1150	0.1070	0.1211	0.1210	0.1271			
KQ072168		0.3053	0.3104	0.3107	0.3184	0.3104	0.3139		
KQ072169	Human	2.7122	2.5971	2.6595	2.8889	2.5370	2.6246	2.7045	
KQ072170		2.8324	2.7191	2.7261	3.0101	2.7767	2.7586	2.8304	0.0129

Discussion

Abortion is the inability to retain the fetus and the loss of the fetus (alive or dead) before the birth date, unlike a miscarriage, which may be known as the spontaneous loss of the fetus.[11] Many agents, such as viruses, bacteria, fungi, and animal parasites, can cause abortion. N. caninum is one of these agents.[12]

Endogenous transplacental transmission occurs when a persistently infected mother experiences a resurgence of the infection during pregnancy. In contrast, exogenous transplacental transmission takes place when a pregnant mother becomes infected after ingesting sporulated oocysts, leading to the initial infection that can then affect the developing fetus).[13] Research has identified that the predominant route of parasite transmission occurs through endogenous transplacental transmission.[14]

In the case of livestock, particularly sheep and goats, endogenous transplacental transmission of *Neospora caninum* is notably effective. Research indicates that this form of transmission occurs in approximately 96.6% of pregnancies in sheep, while in goats, the vertical transmission rate ranges from 71.4% to 100%, which highlights the significant impact of reactivating latent infections during pregnancy on the spread of this parasite among these species.[15]

The mother's immune system is the personal guardian of the fetus, as it often modifies its responses to preserve the fetus. However, despite this, many breakthroughs can facilitate the passage of parasites through the placental hematopoietic pathway to the fetus, especially parasites that can penetrate the walls of the placenta, such as members of the Apicomplexa division.[16] Although the epidemiological, immunological and pathogenetic concepts of human *Neosporosis* infection are not fully understood, the unsuccessful isolation of *N. caninum* from maternal tissues does not mean the absence of vertical transmission of infection from mother to fetus, especially with the presence of molecular evidence of infection of the mother and the presence of the parasite. Therefore, the development of the disease and the severity of infection can largely depend on the age of the pregnant woman and the number of previous parasite infestations.[17]

The arrival of *N. Caninum* in the blood of the umbilical cord necessarily means passing through the placenta. Therefore, this reveals the possibility of the parasite on the human being, even if it is without pathological signs,[18] because the human being is not far from the ways of the horizontal transmission of the parasite, meaning that the injury is very possible. Experimental studies outside the live body have indicated the possibility of the cervical cells and trophoblast cells.[19] It is necessary to stop at the inability of the parasite to settle in human tissues (specifically the placenta), as the parasite's ability to penetrate the host's tissues depends on the delicate balance between the strength of the host's immune system (varying amounts of cytokines and vital cells are produced)[20] and the parasite's possession of the means of adhesion to the host's cell wall, penetration and rapid ability To reproduce inside the cells and then safely switch to the static phase (Bradyzoite).[21] The absence of this balance means that the tachyzoite phases pass quickly to the fetus through the blood path in the umbilical cord.[22]

The affinity between the receptors located on the surfaces of the host and parasite cells enables the adhesion and attachment of the parasite to the surface of the host cells. This association can induce the production of protein microneme contents and thus facilitate the invasion of host cells. The presence of a sufficient number of these receptors and binding signals increases the likelihood of infection and invasion.[23]

Conclusion

The pathogenic effectiveness of the parasite varies depending on the type of host, the duration and size of the infection, so the hosts differ in their response to the infection and their ability to resist the parasite immunologically.

The parasite can infect humans and can cause serious damage, such as miscarriage, despite its inability to cyst inside the placental tissue.

Authors' declaration

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Furthermore, any Figures and images that are not mine have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Ministry of Education.

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التشخيص الجزيئي لطفيلي Neospora caninum في مشايم النساء المجهضات ودم الحبل السري

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الخلاصة

العلاقة بين الإصابة بالبوغية الكلبية في البشر ودورها المحتمل في التسبب في الإجهاض لا تزال محل شك. هذه دراسة تحليلية جزيئية لطفيلي Neospora Caninum في المشيمة ودم الحبل السري من النساء المجهضات. و مقارنة النتائج مع الفحوصات الجزيئية لمشايم عدد من الماشية المجهضة. تم جمع 157 عينة من المشايم من الأبقار المجهضة (58) ، والأغنام (51) ، والماعز (48). بالإضافة إلى ذلك ، تم جمع 115 عينة من الأجنة من (الأبقار (42) ، والأغنام (40) والماعز (33)). أيضًا ، من النساء المجهضات ، تم جمع 45 عينة من المشيمة و 45 عينة من دم الحبل السري من محافظات المثنى والقادسية في منطقة الفرات الأوسط في العراق. كان معدل العدوى الكلي 5,45 ٪ ، 11 من أصل 202 عينة مأخوذة من الحيوانات والنساء كانت إيجابية للعدوى. وقد لوحظت أعلى معدلات العدوى في الحيوانات المسنة، لم يتم العثور على آثار N. caninum في عينات الحمض النووي المستخرجة من المشيمة عند النساء، ومع ذلك ، كان الحمض النووي للطفيلي متواجدًا في دم الحبل السري في 4,4 ٪ من الأجنة البشرية ، كما تم اكتشافه في 2 من 45 عينة. يشير وجود N. caninum في دم الحبل السري إلى أن الأم قد تراكمت فيها العدوى، ومع ذلك ، قد يكون الغياب في المشيمة بسبب الأنواع الفرعية الطفيلية ، والتي قد لا تكون قادرة على اختراق جدران المشيمة ، أو قد يكون الجهاز المناعي البشري له دفاعات تمنع تكوين الطور سريع التكاثر.

الكلمات المفتاحية: البوغية الكلبية، المشيمة، الحبل السري، الاجهاض، جين ن-5.