

Epidemiological survey of abortion in camels (*Camelus dromedarius*) and histopathological investigation of Toxoplasmosis and Neosporosis in those animals from slaughterhouses in Southeastern Algeria

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Abstract

Toxoplasma gondii and *Neospora caninum* are heteroxenous apicomplexan parasites recognized as major causes of reproductive disorders in numerous mammalian species. The present study aimed to assess through an epidemiological survey, the prevalence of reproductive losses in 75 dromedary herds during the period 2022-2025, as well as the associated risk factors, and to detect these protozoa by histopathological analysis in 63 dromedaries slaughtered at the slaughterhouse of El Oued province (126 samples: 63 hearts and 63 diaphragms). The survey revealed that 80% of the herds had a history of abortions, of which 60% also reported cases of stillbirth. Risk factor analysis showed a significant association exclusively with the presence of domestic cats and dogs ($P < 0.05$). Histological examination revealed forms compatible with *T. gondii* exclusively in the cardiac muscle of adult females, with tachyzoites detected in one animal (1.6%) and tissue cysts in two animals (3.17%). No evidence of *N. caninum* was detected. Cardiac lesions were characterized by mononuclear cell infiltrates, focal hemorrhages and areas of necrosis, indicating marked inflammatory and vascular reactions. These findings suggest that *T. gondii*, may be involved in reproductive losses in dromedaries and effective parasite control strategies specific to dromedary camels need to be developed.

Keywords: abortion, *Toxoplasma gondii*, *Neospora caninum*, dromedaries, histopathology

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Introduction

Toxoplasma gondii and *Neospora caninum* are cyst-forming coccidian protozoa with a worldwide distribution. These pathogens are major causes of embryonic death and fetal resorption, fetal death and mummification, abortion, stillbirth, and neonatal death in herbivorous livestock, thereby causing substantial economic losses in livestock production system (Djeddou-Benabid *et al.*, 2025; Houssou *et al.*, 2025). Their lifecycle is heteroxenous, involving a sexual phase with oocyst production in the intestines of definitive hosts (*T. gondii* in domestic and wild felids, *N. caninum* in dogs, wolves, coyotes, and dingoes) and an asexual phase occurring in the extraintestinal tissues (tissue cysts) of intermediate hosts (Dubey and Lindsay, 2006). There are three different infectious stages: sporozoites within oocysts, tachyzoites, and bradyzoites present in tissue cysts (de Moraes *et al.*, 2011). The transmission of these protozoan parasites in herbivorous livestock can occur via two main routes: horizontally or vertically. In addition, carnivorous and omnivorous species may become infected by ingesting tissues from intermediate hosts containing viable cyst stages (Basso *et al.*, 2022). Due to their microscopic size, routine detection of *T. gondii* and *N. caninum* cysts during meat inspection is not feasible. Consequently, the diagnosis of *T. gondii* and *N. caninum* infection relies primarily on serological approaches. Indeed, according to Fereig *et al.* (2022), the global seroprevalence of *T. gondii* and *N. caninum* in the Camelidae family has been estimated at 28.16% and 14.3%, respectively. Although no microscopic identification of tissue cysts was reported, molecular detection methods (PCR) have been applied to cardiac and diaphragmatic muscle samples in studies conducted in Egypt, where prevalence rates of 55.5% (2017) and 1.1% (2019) were reported, as well as in Iran, with prevalence rates of 65% (2016) and 13% (2018) (Fereig *et al.*, 2022). Furthermore, Tonouhewa *et al.* (2017) estimated the overall seroprevalence of toxoplasmosis in dromedaries (*Camelus dromedarius*) in Africa to be 36% (18% to 56%). However, this serological evidence was not supported by direct detection or microscopic examination of *T. gondii* cysts in muscle tissues.

Abortions caused by *T. gondii* and *N. caninum* have been widely documented in small ruminants and cattle, respectively (Dubey and Lindsay, 2006). In contrast, abortions attributed to these two protozoa are rare in camelids. Nevertheless, a few clinical cases have been reported: abortions associated with *N. caninum* infection have been described in alpacas and llamas (Serrano-Martínez *et al.*, 2007), while an abortion attributed to *T. gondii*, associated with hemorrhagic colic and toxoplasmic peritonitis, was recently reported in a Bactrian camel (Kommenou *et al.*, 2022). In dromedaries (*Camelus dromedarius*), only a single case of acute toxoplasmosis has been reported, associated with abortion and clinical signs in the animal (Hagemoser *et al.*, 1990).

To date, no study has reported the microscopic detection of *T. gondii* and *N. caninum* in dromedary camels in Algeria. Therefore, the present study aimed to (i) assess the incidence of abortion in dromedaries

through a breeder-based survey, and (ii) perform a histopathological analysis to identify *T. gondii* and *N. caninum* in selected tissues from slaughtered dromedaries in Southeastern Algeria.

Materials and Methods

Ethics statement: All procedures adhered to the guidelines and regulations of the Institutional Committee for Animal Protection under the Algerian Ministry of Higher Education and Scientific Research (Decree No. 98-11 of August 22, 1998).

Study area: The study was conducted in El-Oued Province, located in Southeastern Algeria (33°21'21" N, 6°51'47" E). Covering an area of 44,586.80 km², El Oued is one of the largest administrative regions in the country, occupying a strategic position and serving as a major center for dromedary breeding and supply in Southeastern Algeria. The region is characterized by a hot desert climate (Köppen-Geiger classification BWh), with annual temperatures ranging from an average minimum of 21.8°C to a maximum of 39°C, a mean relative humidity of 24.3%, and scarce rainfall averaging between 80 and 100 mm per year.

Questionnaire survey: An epidemiological survey was conducted among 75 dromedary breeders across the entire El Oued province using a structured questionnaire. The aim was to collect information on:

- Herd management (Farms were classified into three systems : extensive system, where herds primarily grazed on natural rangelands with minimal external inputs, semi-intensive system, where herds were managed using a combination of controlled grazing and supplementary feeding and intensive system, where herds were kept in specialized units with high levels of inputs, including feed supplementation and veterinary care, often for fattening or racing purposes) (Mekhloufi, 2020).

- Reproductive losses (history of abortions and stillbirths during the periods 2022/2023 and 2024/2025). Abortion frequency (%) was calculated by dividing the number of females that aborted by the total number of females in the herd. It was then classified according to the thresholds defined by Givens and Marley (2008) in cattle: high rate (>5 %), frequent (2-5 %), and rare (<2 %).

- The presence of other animal species (cattle, sheep, and goats), as well as the presence of cats and dogs. The breeders were randomly selected.

Histopathological analysis: This study was conducted in February 2025 on 63 clinically healthy dromedaries, including 23 males and 40 females, aged between 12 months and 15 years. All animals were slaughtered at the slaughterhouse (Royal) located near the capital of El Oued province.

A total of 126 samples were collected (63 hearts and 63 diaphragms). Cardiac and diaphragmatic tissue fragments, approximately 50 g each, were collected immediately after opening the abdominal cavity of the slaughtered animals and fixed in 10% neutral-buffered formalin for at least 24 hours. The fixed samples were

trimmed, dehydrated through a graded ethanol series, cleared in xylene, infiltrated, and embedded in paraffin wax. From each block, tissue sections of 4 to 5 μ m thickness were prepared using a rotary microtome. The slides were then deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E).

The slides were examined using a light microscope (OPTIKA B-293PLi, Italy, 10 $\times$ and 40 $\times$ objectives), equipped with a motorized stage and a digital camera. The entire system, connected to a microcomputer, enabled automated image acquisition through sample scanning, ensuring accurate documentation of histopathological findings.

Statistical analysis: The questionnaire survey data were recorded and coded in a Microsoft Excel spreadsheet (Microsoft Corporation) and then analyzed using the SPSS software (version 27). Herd-level abortion prevalence was defined as the proportion of herds reporting at least one abortion during the reproduction period and was calculated by dividing the number of positive herds by the total number of herds surveyed. Associations between this prevalence and potential risk factors were assessed using Chi-square and Fisher's exact tests. A 95 % confidence level was adopted, and $P < 0.05$ was considered statistically significant.

Results

Profile of the studied herds: The three farming systems (extensive, intensive and semi-intensive) are represented by 62.7%, 9.3% and 28.0%, respectively.

Among the 75 breeders surveyed, 80% (60/75) reported a history of abortion. Of these 60 breeders, 60% (36/60) also reported cases of stillbirth.

Among the 60 herds, 8.3% (5/60) experienced high abortion rates, 40% (24/60) experienced frequent abortions, and 51.7% (31/60) experienced rare abortions.

In the sixty camel breeders that reported a history of abortion, the presence of other animal species was also considered, including cattle 5% (3/60), sheep 58.3% (35/60), goats 60% (36/60), dogs 71.6% (43/60), and cats 80% (48/60). Furthermore, 13 herders also reported the possibility of contact with stray dogs and cats.

Correlation analysis and risk factors: Chi-square test: Table 1 presents the results of the analysis of potential risk factors associated with herd-level abortion prevalence in female dromedaries in El Oued province.

Among the variables studied, two factors were found to be significantly associated with the occurrence of abortions: the presence of cats and the presence of dogs ($P < 0.05$). In contrast, no statistically significant association was observed for the other factors analyzed, including geographical location, cohabitation with cattle or small ruminants (sheep and goats), and herd management.

Table 1 Analysis of potential risk factors associated with abortion in female dromedaries.

Variables	Categories	N. of SH	N. of HA	(χ^2)	P- value
Locality	Taleb Larbi	1	1	16,485	0.170
	El Rabah	6	3		
	Mih Ouensa	20	16		
	El Ogla	3	3		
	Hassi Khalifa	7	6		
	El Oued	3	1		
	Magrane	5	5		
	Bayadha	2	1		
	Oued El Alenda	6	4		
	Reguiba	5	5		
	Benguecha	13	11		
	Douar El Ma	2	2		
	Nakhla	2	2		
The presence of cattle	No	68	57	0.425	0.514
	Yes	7	3		
The presence of sheep	No	35	25	0.044	0.833
	Yes	40	35		
The presence of goats	No	31	24	0.044	0.833
	Yes	44	36		
The presence of dogs	No	26	17	7.520	0.006*
	yes	49	43		
The presence of cats	No	23	12	4.271	0.039*
	Yes	52	48		
Husbandry system	Extensive	45	35	2.394	0.302
	Intensive	7	3		
	Semi-intensive	23	22		

N: Number; (χ^2): Pearson Chi-Square; SH: Surveyed herds; HA: herds reporting abortions.

* Statistical significance.

Detection of *T. gondii* and *N. caninum* using histopathological analysis: A total of 126 tissue samples, including 63 cardiac muscle and 63 diaphragmatic muscle samples, were examined histopathologically. Parasitic structures morphologically compatible with *T. gondii* were detected exclusively in the cardiac muscle of three female dromedaries (3/63; 4.8% of the examined animals). No cysts were observed in the diaphragmatic samples. The positive animals corresponded to samples No. 11, No. 12, and No. 60, aged 10, 12, and 11 years old, respectively.

Tachyzoites were observed in sample No. 11 (1/63; 1.6% of the examined animals) (Fig. 1A), whereas tissue cysts containing bradyzoites were identified in samples No. 12 and No. 60 (2/63; 3.17% of the examined animals) (Fig. 1H and Fig. 1D, respectively).

The identification of *T. gondii* cysts in HE-stained sections is challenging due to their morphological similarity to other protozoa such as *N. caninum* and *Sarcocystis* spp. The tissue cysts observed in the cardiac muscle (Fig. 1 D–H) exhibited a regular structure, measuring 72 and 100 µm, respectively. They were surrounded by a thin and uniform cyst wall (<0.5 µm), morphological features consistent with *T. gondii*. In the differential diagnosis, cysts of *N. caninum* exhibit a thicker and more irregular wall (0.5–4 µm), whereas those of *Sarcocystis* spp. vary in size and shape and are characterized by a cyst wall with hair-like projections or radial striations (Dahmani et al., 2018; Dubey and Lindsay, 2006).

An inflammatory response was associated with parasite presence in two cases: sample No. 11 showed a cardiac inflammatory infiltrate composed of mononuclear cells (Fig. 1B), and sample No. 60 exhibited marked inflammatory lesions characterized by mononuclear cell infiltrates, focal hemorrhages, and areas of necrosis (Figs. 1C and 1E–G). In contrast, no inflammatory reaction was observed in sample No. 12 despite the presence of tissue cysts. No inflammatory lesions were detected in the diaphragmatic muscle of any of the examined animals.

Furthermore, no parasitic elements compatible with *N. caninum* were identified in either the cardiac or diaphragmatic muscle analyzed.

Discussion

History of abortion and related risk factors: Among the surveyed breeders, 80% reported having observed abortions in their herds over the past four years, reflecting a high prevalence of affected herds during the study period. These findings confirm previous observations reported by Khalafalla et al. (2017). Indeed, abortions can have a major impact on reproductive performance, leading to an extension of the calving interval, which may increase from the usual average of 24 months up to 36 months (Faye et al. 2023). Although the majority of herds exhibited a low frequency of abortions, some recorded a higher frequency, reflecting their vulnerability and highlighting the need for targeted monitoring and appropriate preventive measures. Early abortions are difficult to detect, and observed rates are therefore likely underestimated (Henker et al., 2022). According

to Tibary et al. (2006) abortion rates in dromedaries range from 2% to 25%, classifying them as highly significant. The stillbirth rate observed in the studied herds is comparable to that reported by Nagy et al. (2023). These findings suggest a strong correlation between abortion and stillbirth cases in dromedaries, reflecting the likely influence of persistent infectious agents or environmental factors on gestation and neonatal survival. The analysis of correlations between herd-level abortion prevalence and various variables revealed statistically significant associations, particularly with the presence of cats and dogs, suggesting that these domestic carnivores may play a key role in the transmission of infectious agents such as *T. gondii*, *N. caninum*, which can affect dromedary reproduction.

Histopathological study: Data on the presence of *T. gondii* and *N. caninum* in the tissues of naturally infected dromedaries in North Africa remain limited. To our knowledge, this study represents the first combined histopathological report of these two protozoa in dromedaries slaughtered in Algeria, documenting their presence in cardiac muscle and providing baseline data for future investigations on their potential role in reproductive health.

Histological examination of H&E stained sections of the organs studied (heart and diaphragm) revealed forms compatible with *T. gondii* exclusively in the heart. No forms of *N. caninum* were detected in either the heart or the diaphragm. It should be noted that *N. caninum* tissue cysts are rare, and compared with *T. gondii*, their numbers are very limited in naturally infected animals such as cattle and other intermediate hosts (Speer et al., 1999). The absence of histopathological detection of *N. caninum* in our study is consistent with the observations of Amdouni et al. (2022), who reported that microscopic examination of the brain, tongue, and masseter muscle of infected dromedaries was negative, despite a molecular prevalence of 34.3% (12/35). In contrast, Karimi et al. (2023) identified suspected *N. caninum* stages in the brain of a single dromedary, which also tested PCR-positive in the brain, blood, and lymph nodes. To date, only two studies have reported the isolation of *T. gondii* from naturally infected dromedaries: in Saudi Arabia (Hilali et al., 1995) from the tongue and esophagus, and in Ethiopia (Gebremedhin et al., 2014) from the heart and tongue, with cysts isolated in 16.13% (5/31) of DAT-positive animals. In Algeria, no data are available regarding the presence of this parasite in dromedary tissues. However, a single histopathological study conducted in sheep detected *T. gondii* cysts in the heart and diaphragm, with a very low prevalence of 0.59% (2/335) (Dahmani et al., 2018). The absence of *T. gondii* or *N. caninum* in one or both organs (heart, diaphragm) may be explained by the inherent difficulties in detecting these parasites in tissue sections from large animals. These challenges are related to the limited sample size, low parasite density, and the restricted sensitivity of microscopy, with the pathogen potentially residing in unexamined areas (Esteban-Redondo et al., 1999; Gebremedhin et al., 2014). Moreover, a negative histological result does not necessarily indicate that the entire tissue is free of the

parasite (Nunes *et al.*, 2015). It should also be emphasized that the low proportion of positive cases

(3 out of 63 subjects examined) may represent a limitation of the study.

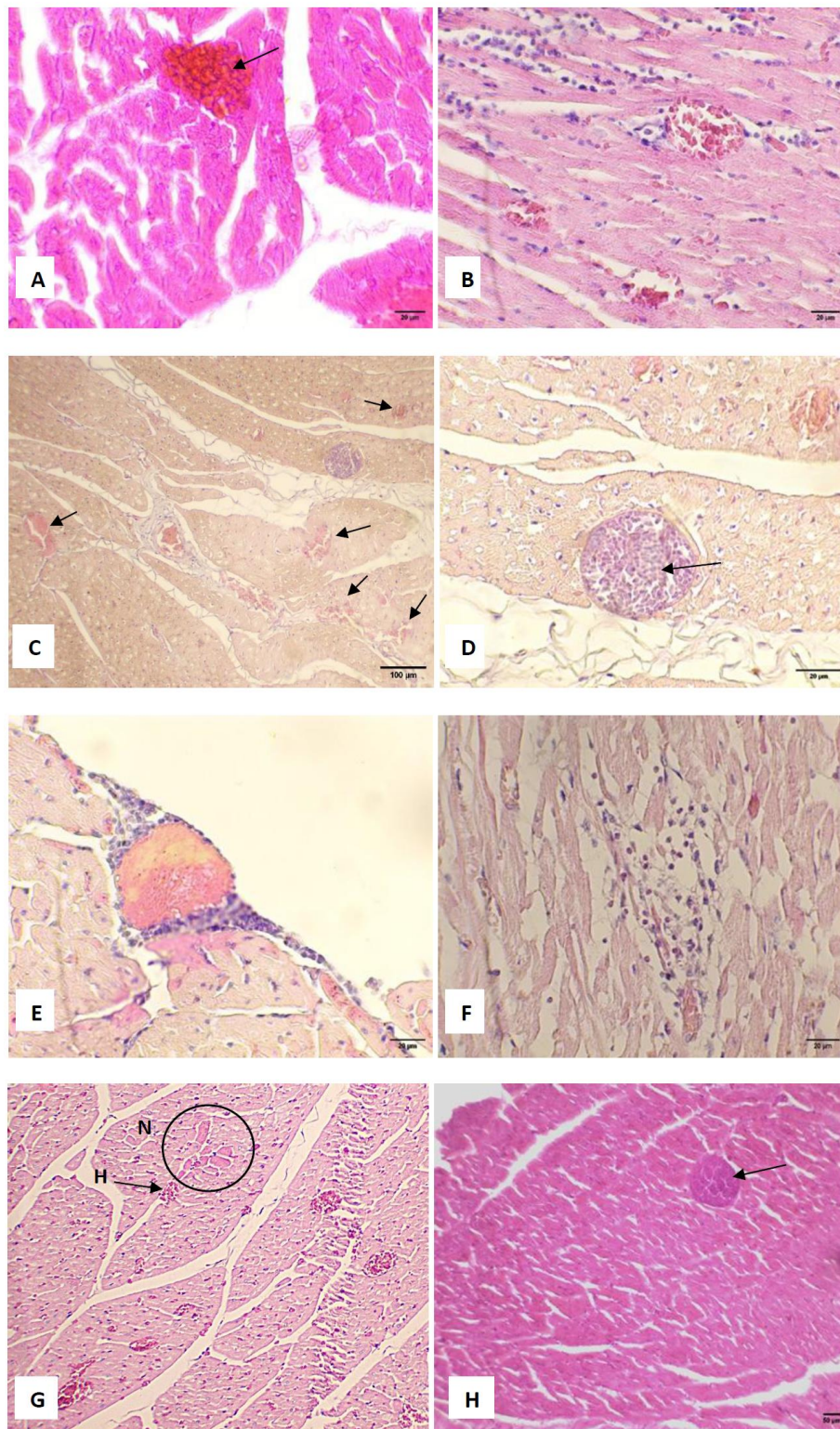


Figure 1 Cardiac lesions and detection of *Toxoplasma gondii* in histological sections of the heart of naturally infected dromedaries (A-I hematoxylin and eosin). A. Dense cluster of *T. gondii* tachyzoites within cardiac muscle fibers (arrow). B. Mononuclear interstitial inflammatory infiltrate consistent with focal active myocarditis. The massive infiltration of inflammatory cells within the muscle fibers is manifested by numerous nuclei stained dark purple. C. Tissue cyst of *T. gondii* with diffuse microvascular congestion of blood vessels (arrows). D. Detail of a toxoplasmic cyst $\times 400$. Note the circular, well-defined outline of the cyst and the numerous *T. gondii* bradyzoites within (arrows). The cyst size is estimated at approximately 72 μm , cyst wall ($<0.5 \mu\text{m}$), scale bar= $20 \mu\text{m}$. E. Marked interstitial and perivascular lymphoid infiltrate surrounding a congested venule within the cardiac tissue $\times 400$, scale bar= $20 \mu\text{m}$. F. Focal lymphohistiocytic infiltration in the tissue characterized by an aggregation of mononuclear inflammatory cells. G. Moderate necrosis (N) and hemorrhage (H) in cardiac tissue $\times 100$, scale bar= $50 \mu\text{m}$. H. *T. gondii* cyst without inflammation or tissue lesions (arrow) $\times 100$, scale bar= $50 \mu\text{m}$.

Parasitic involvement was strictly confined to the cardiac muscle, with tachyzoites detected in one animal (1.6% of the sampled subjects) and tissue cysts in two animals (3.17%). The identified cysts (Figs. 1 D-H) exhibited a regular and well-defined structure, with a thin cyst wall enclosing numerous bradyzoites, measuring 72 and 100 μm , respectively. According to Dubey *et al.* (1998), a mature tissue cyst of *T. gondii* is generally spherical in the brain and measures on average 50-70 μm in diameter, whereas in muscle tissues, cysts are typically more elongated and may reach up to 100 μm in length. More broadly, the size of tissue cysts varies depending on cyst age, the type of infected host cell, the *T. gondii* strain, and the cytological method used for measurement. Other authors have also reported toxoplasmic cysts measuring up to 100-200 μm (Desoubaux *et al.*, 2012). The cardiac tropism observed in dromedaries may reflect a preference of the parasite for the heart, without excluding its ability to reach other tissues. It should be emphasized that the presence of tachyzoites and bradyzoites may reflect different stages of infection and is closely associated with the tissue tropism of *T. gondii* within each organ (Kılınç *et al.*, 2023). During the acute phase of infection, the parasite can rapidly cross intestinal epithelial cells and reach various cell types in different tissues via the bloodstream and/or lymphatic circulation (Tenter *et al.*, 2000). In contrast, cystic forms predominate during chronic infection and are more frequently observed in nervous tissues (brain and retina) as well as in muscular tissues, notably skeletal and cardiac muscles (Dubey and Lindsay, 2006). Experimental studies have shown that at high infectious doses, *T. gondii* is more frequently and consistently detected in the brain and heart, suggesting that these organs represent preferential sites for tissue localization in animals (Esteban-Redondo *et al.*, 1999; Kılınç *et al.*, 2023). Toxoplasmic cysts were detected in the heart of adult dromedaries in the study by Gebremedhin *et al.* (2014). Apart from this research, no published histopathological study has definitively confirmed the presence of cysts in the cardiac muscle or diaphragm of dromedaries, alpacas, or llamas. Nevertheless, several studies in ruminants and other species indicate that these tissues serve as preferential sites for cyst persistence. In domestic ruminants, *T. gondii* cysts have been detected in the cardiac muscle of seropositive buffaloes and sheep, whereas their presence in the diaphragm appears to be less frequent (Gareh *et al.*, 2025; Tenter *et al.*, 1992; Zghair, 2016). These observations are fully consistent with our results and indicate that *T. gondii* shows a marked predilection for the cardiac muscle over skeletal muscles, although cysts can also be detected in the latter, particularly under natural infection conditions (Hussein *et al.*, 2023). Studies in birds of prey have also reported *T. gondii* cysts in the myocardium and skeletal muscles, confirming the parasite's ability to persist in these tissues (Elmore *et al.*, 2010; Zanet *et al.*, 2023). For *N. caninum*, thin-walled cysts have been described in the muscles of naturally infected cattle and dogs, although their detection in experimentally infected animals remains limited (Dubey and Lindsay, 2006; Peters *et al.*, 2001). Therefore, the cardiac and skeletal muscles

represent potential reservoirs for the persistence and transmission of both parasites.

In the present study, all dromedaries in which tissue forms of *T. gondii* were identified were female Sahraoui camels aged 10 years or older. These observations suggest that, in older females, chronic infection associated with the presence of tissue cysts may constitute a risk factor for parasite reactivation during gestation, thereby facilitating transplacental transmission to the fetus and increasing the risk of abortion. This association between advanced age, chronic infection, and adverse reproductive outcomes is supported by studies in other ruminants, where chronically infected females could transmit the parasite to the fetus, resulting in abortion or stillbirth (Dubey, 2009; Innes *et al.*, 2007).

Significant histopathological lesions were observed exclusively in the heart. These lesions were characterized by a mononuclear cell infiltrate, associated or not with a perivascular inflammatory cuff. The alterations were also accompanied by venular congestion, focal hemorrhages, and areas of necrosis, indicating a marked inflammatory and vascular response in the cardiac tissue. According to Nunes *et al.* (2015), the intensity and nature of histological changes may vary between studies, particularly regarding the extent of the mononuclear infiltrate in tissues targeted by the parasite. These authors specifically reported that the main histopathological lesion observed in the heart consisted of a mononuclear cell infiltrate. Desoubaux *et al.* (2012) also described that the influx of inflammatory cells within cardiac muscle fibers is manifested by the visualization of their nuclei stained dark purple within an interstitial and perivascular lymphoid infiltrate. Furthermore, Dubey *et al.* (1998) reported that intact tissue cysts likely cause no tissue damage and can persist without inducing a host inflammatory response, whereas severe inflammation accompanied by local necrosis occurs when cysts rupture and release the parasites. Consistent with our findings, Machado *et al.* (2016) also demonstrated that experimental *T. gondii* infection leads to cardiac alterations, histopathologically characterized by necrosis, hemorrhagic areas, and inflammatory infiltrates.

To date, no data have established a direct link between toxoplasmosis or neosporosis and abortions in dromedaries (*Camelus dromedarius*), with the exception of a single case of acute toxoplasmosis reported by Hagemoser *et al.* (1990). It involved a 6-year-old female that had aborted a near-term fetus, presenting with anorexia and dyspnea, in which *T. gondii* tachyzoites were detected in pleural macrophages and anti-*T. gondii* antibodies were strongly positive, confirming the diagnosis. It is important to note that infection with *T. gondii* or *N. caninum* has long been recognized as a potential cause of abortion in alpacas and llamas (Pearson *et al.*, 2014). In these two camelid species, *N. caninum* DNA as well as various parasitic stages have been identified in the brain and heart of aborted fetuses using histology, immunohistochemistry, and PCR techniques (Serrano-Martínez *et al.*, 2007). Furthermore, Dubey *et al.* (2014) reported the first confirmed case of abortion associated with toxoplasmosis in an alpaca. In this context, tissue

cysts of *T. gondii* were detected in the kidneys and brain of the fetus, while mild inflammatory lesions were observed in the placenta, liver, lungs, and brain, including nonsuppurative meningoencephalitis, hydrocephalus, and hydromyelia. Given the limited sensitivity of histology, it is recommended to combine it with other techniques, particularly serological and molecular methods, in order to more reliably assess the contribution of these two protozoa to abortions, while also providing a better understanding of their tissue distribution and tropism.

In conclusion, the detection of *T. gondii* tissue forms in adult female dromedaries, together with the observed association between herd-level abortion history and the presence of domestic carnivores (cats and dogs) in the study area, suggests that *T. gondii* may be a contributing factor to reproductive losses in Algerian dromedaries. However, as no direct causal link was established between infected animals and previous abortion events, it is likely that other factors, such as environmental conditions, herd management practices, or exposure to other infectious agents, also play a significant role. These findings highlight the susceptibility of dromedaries to protozoan infections and underscore the need for integrated reproductive health management approaches, combining parasite control with improved husbandry practices.

Declaration of generative AI

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) exclusively for superficial language editing purposes (including grammar and spelling correction). The authors reviewed, verified, and approved the entire manuscript and take full responsibility for the final content of this publication.

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