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Molecular Detection of *Toxoplasma gondii* Parasite in Cow, Buffalo, and Camel Milk

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Abstract | *Toxoplasma gondii* is an obligate intracellular parasite and is a rather common and widely spread parasite of warm blood mammals (including humans), birds and cats (Felidae) (felines). However, the definitive hosts of *Toxoplasma gondii* are felines, while warm blooded mammals and birds are the intermediate hosts. Contaminating livestock products is an infection threat to public health due to this zoonotic infection. In particular, it is also one of the most crucial problems threatening human health due to raw meat and unpasteurized milk. In order to assess the prevalence and molecular detection of *Toxoplasma gondii*, a total of 150 milk samples (50 samples virtuose of cow milk, 50 samples virtual of buffalo milk and 50 samples of camel milk) were collected randomly including and by direct milking the animal. Through examination of all milk samples using molecular technique reveal 25 samples (16.66%) were positive for the *Toxoplasma gondii* parasite, and were distributed in buffalo milk (14%), cow milk (16%), and camel milk (20%) in ascending order. The results of the current study revealed that cow, buffalo, and camel milk could be an important route for the transmission of the *Toxoplasma gondii* parasite to humans through the consumption of raw milk as food.

Keywords | *Toxoplasma gondii*, Al-Diwaniyah, Milk, Cow, Buffalo, Camel

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INTRODUCTION

The milk is considered complete food due to abundant proteins, minerals, lipids and vitamins in milk. The nutritional values of milk have revitalized early human life from baby to adult livelihood. Cow's milk contains calcium and casein in abundant amount than human milk, while buffalo milk carries more cholesterol than cow's and camel's milk. Cow's milk has a higher iron and vitamin C than camel's milk (López-Soto *et al*., 2010; Boughattas, 2017). Socially, it appeared that people favored cow's milk, buffalo milk as well as camel milk when compared to sheep and goat milk and people who prefer such milk are approximately half of the population that consumes such milk raw or boiled (Boughattas, 2017).

An example of the obligate intracellular parasite in the Apicomplexa class in the Protozoa phylum is *Toxoplasma gondii*, an organism which can infect all nucleated cells (Webster, 2010). The parasite is present in milk produced from camels, cows, buffaloes, sheep or goats. Therefore, an estimated incidence of 244.1 cases per 1000 infected camels, all of them with serious insufficiency of immunity due to the widespread dispersion of *T. gondii* infection strain in their livestock can be reported. The economic loss inflicted by toxoplasmosis on breeders of sheep, goats,

cattle, buffalos and camels, due to abortion is enormous. This disease compromise animal productivity and work to create worrisome public health outcomes (Wang and Meng, 2020). Unpasteurized milk drinking might enhance chance of getting a *Toxoplasma gondii* infection and raw meat and unpasteurized milk are major contributors to infection (Pagmadulam *et al*., 2018; Aguirre *et al*., 2019). Toxoplasmosis also causes a person to not lactate as adequately or to be infertile for an extended period of time (Liyanage *et al*., 2021).As noted by previous studies (Chiari and Neves, 1984; Remington *et al*., 2004; Ragozo *et al*., 2009; Camossi *et al*., 2011), stages of rapid division of *T. gondii* have been found in the milk of sheep, goats, cattle and mice. In Brazil, it has been documented that the parasitic DNA was identified in goats, Other studies (De Santana *et al*., 2015) identified *T. gondii* in several animal species in Brazil, (Saad *et al*., 2018) studied the parasite in goats, sheep and camels in Egypt, sheep in Italy (Fusco *et al*., 2007), people and goats in Iran (Khamsian *et al*., 2021), and in cows in China (Wang and Meng, 2020). Sheep were studied in Egypt (Fereig *et al*., 2022), goats in Iraq (Razooqi *et al*., 2022), and both sheep, goats, and buffaloes in Pakistan (Khan *et al*., 2023). Additionally, camels, cows, and buffaloes were studied in Iran (Aghdam *et al*., 2023). Several experimental studies have confirmed that milk from animals infected with the parasite *Toxoplasma gondii* contains rapidly reproducing forms (Chen *et al*., 2021). These fast-reproducing forms have been found and isolated from the milk of goats. In certain parts of the world, milk plays a role in the transmission of *Toxoplasma gondii* infection to humans (Fereig *et al*., 2022).

This study highlights the importance of the information about potentially transmitting *Toxoplasma gondii* through cow, buffalo and camel milk, especially if the milk is unpasteurized in rural and nomadic areas in general, the aim of this study is to investigate the presence of *Toxoplasma gondii* in cow, buffalo, and camel milk samples. We attempted to collect raw milk and performed molecular detection using will be done through polymerase chain reaction (PCR) followed by the examination of the genomic DNA of Cox B1 gene of the *T. gondii* parasite in raw milk. Our finding contributes to the epidemiological investigation of the parasite in the governorate and Iraq in general.

MATERIALS AND METHODS

Sample collection

Randomly, milk samples were collected from animals between 15/9/2024 and 8/2/205 from cows, from buffaloes and camels; A total of 150 milk samples were collected and these were randomly and equally distributed in 50 cows (n=50), buffaloes (n=50) and camels (n=50),The following equation was used to calculate sample size examine in order to get an accurate estimate of the parasite prevalence with a high confidence level. If we assume 96% confidence level and 5% margin of error 0 as outlined before (Aghdam *et al*., 2023) and the prevalence of the *T. gondii* parasite in animal milk is 5%-10% based on previous studies, then the range of prevalence of cross infections in the milk can be calculated as:

$$n = \frac{z^2\,\alpha/2\text{-}1 \times p(1-p)}{^2d}$$

Where: n: z2α / 2-1: The z-statistical value at required confidence level (i.e. 96%), p: The estimated Prevalence *T. gondii* in animal milk (5-10 percent), P-1: The remainder (difference) of uninfected animals, and d: The acceptable margin of error (5 percent). Probable milk from each animal was collected in 10 ml milk sample flasks with gloves and manual milking of the animal directly from its teats, post sterilizing with ethyl alcohol. To conduct a polymerase chain reaction (PCR) test, the parasite's DNA was extracted from the samples refrigerated and transferred to the laboratory using DNA extraction kit.

DNA extraction

10 ml of milk tubes were transferred to and centrifuge for 3 minutes at a speed of 14000/16000 rpm. The sediment was kept, and the upper part (casein) was discarded. The DNA was extracted by a set of steps using a special kit from GeneAid company, Taiwan according to instructions of the manufacturer.

PCR amplification

The B1 gene of the *T. gondii* parasite was amplified using sensitive primers specific to 529 base pairs (Camossi *et al*., 2011; Must *et al*., 2017). (TOX4 -´5) CGCTGCAGGGAGGAAGACGAAAGTTG-3´ (and TOX5 -´5) CGCTGCAGACACAGTGCATCTGGATT-3´). The PCR reaction mix include DNA extract four µl, forward primer three µl, reverse primer four µl, distilled water DNS free fifteen µl, master mix 25 µl (Taq DNA Polymerase, dNTP (dATP, dCTP, dGTP and dTTP) buffer, loading dye, (MgCl2). The PCR reaction was programmed as one cycle at 94 °C 5 minutes, 35 cycle at 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for 1 minute and final step 72°C for 10 minutes. The seven microliters of PCR product were loaded onto a 1.5% agarose gel. Once the gel was lifted and transferred to the front of an American made UV transilluminator, the PCR products were photographed. All result were then analyzed using SPSS statistical program and Chi-Square test (Daniel and Cross, 2018).

RESULTS

Using 150 milk samples collected from various animals, 25 showed infection of *Toxoplasma gondii* (16.66%). As shown in Figure 1 and Figure 2, there were eight positive samples (16%) in cow milk and 7 positive samples (14%) in buffalo milk samples. The positive samples (20% in 50 camel milk samples) appeared positive in camel milk (Figure 3).

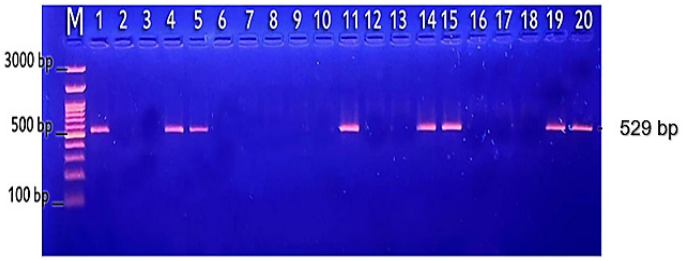

Figure 1: Similarly, agarose gel electrophoresis (1.5) displaying PCR products for the B1 gene in positive cow milk samples, positive samples in columns 1, 4, 5, 11, 14, 15, 19, 20 at 529 base pairs bp molecular length (M: Ladder measurement scale of 100-3000 base pairs).

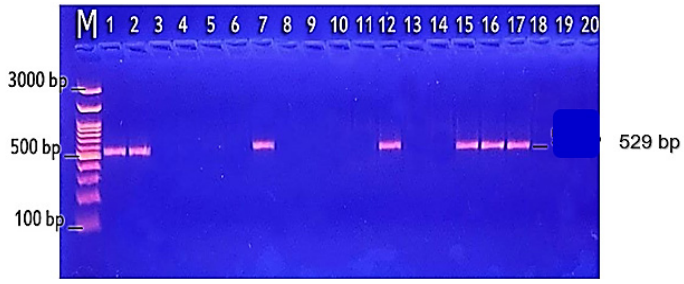

Figure 2: Agarose gel electrophoresis (1.5) PCR products of gene B1 in positive buffalo milk samples as shown in columns 1, 2, 7, 12, 15, 16, 17 with positive samples from molecular length 529 base pairs bp; M; ladder measurement scale in size (100-3000 base pairs).

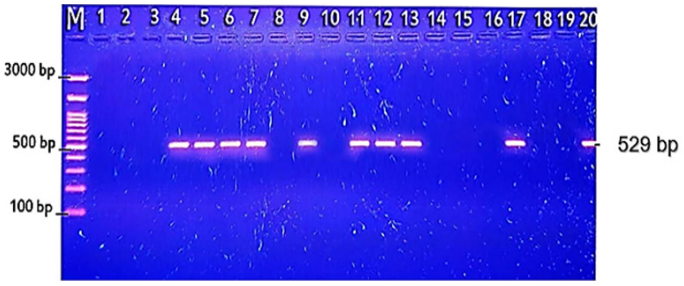

Figure 3: PCR products for gene B1 in positive camel milk samples (1.5) agarose gel electrophoresis display, in columns 20, 17, 13, 4, 5, 6, 7, 9, 11, 12 positive samples at the molecular size of 529 base pairs bp., M: ladder measurement scale at 100-3000 base pairs bp.

No statistically significant differences were observed in the infection rates among animals at probability ($P \leq 0.05$) and the highest positive rate for the parasite was in camel milk (20%) and lowest in buffaloes (14%).

DISCUSSION

In general, the Iraqi economy greatly depends on the products that daily to its internal markets. It can lead to infection in cows, buffaloes or camels, and result in miscarriage or a reduced milk production and hence negatively impact the Iraqi economy and incur economic losses. Meat and milk production, which make it a nutritious and essential product of human consumption, is a great source of meat and dairy products in the economy of many countries, especially Asian countries. In addition, camel milk is also of medical and nutritional value for human consumption in particular among the Bedouins.

Purchasing unpasteurized milk is another main risk of public health and another way to contract toxoplasmosis. This has been proven in many studies: gastric juices can destroy rapidly reproducing stages. But many studies have shown that human beings can become infected despite gastric juices destroying rapidly reproducing stages (Saad *et al*., 2018). The results of this study indicate that camels are more susceptible to infection with *Toxoplasma gondii* parasite than cattle and buffalo in Diwaniyah province. The results of the current study revealed that the milk of cows, buffaloes, as well as camels are contaminated with the *Toxoplasma gondii* parasite. Thus, it has been demonstrated in many different studies that cows act as a resistant host to the *Toxoplasma gondii* parasite, develop a more effective immune response against infection with the *Toxoplasma gondii* parasite, and infection of cows with the *Toxoplasma gondii* parasite may not always result in abortion or deaths near birth (Esteban-Redondo and Innes, 1997; Wiengcharoen *et al*., 2011). This may be accounted for in the type of host or perhaps by the feeding habits of animals (Tavassoli *et al*., 2013).

There is also a study that indicates that it is the number of animals in a herd, the age of the animals raised, or how they are raised that bring about a spread of toxoplasmosis for the amount of infection is less in large farms and also when conditions relating to the place where the animals grew, the final host contact with the animals, and poor methods of storing farm animal food exist (Holec-Gasior, 2013). It was also found that the rates of infection are higher in humid tropical areas than in hot, dry and temperate (Kazem and Ali, 2 024). Results of our study revealed that 16% 14% and 20% cow buffalo and camel milk samples were infected with the parasite respectively. However, a study (Hajipour and Gholami, 2023) found the DNA of *Toxoplasma gondii* parasite in cow, buffalo, and camel milk samples at the amount of 3.36%, 3.33%, and 13.33%, respectively, by polymerase chain reaction. New study from Iran about Prevalence of the parasite in organs of commercial infected youth bucks, and unpasteurized

milk of cows, buffaloes and camels by using the nested polymerase chain reaction technique was 2.38%, 2.10%, and 1.50%, respectively (Abdollahzadeh *et al*., 2022). Our study results showed that camels are more susceptible to toxoplasmosis. One possible reason for camels to be more susceptible to infection as observed in this study as opposed to other previous studies is that oocysts could be present in the soil and ground water from cat feces contamination (Ferguson, 2009). Another reason is also that some breeders don't know well how to deal with animal abortions such as not washing their hands after the treatment of abortions or not healthily throwing them away and leave them along the road (Gebremedhin *et al*., 2016). In addition, some breeders transport goods using the camels and to plow the fields on agricultural fields thus making them more mobile and mobile in the region (Shehzad *et al*., 2022). It is due to the difference in animals breeds and to toxoplasmosis susceptibility patterns and feeding methods (Must *et al*., 2017; Guo *et al*., 2015). Results of this study tallied up with a study conducted on Iran, where the Prevalence of the parasite in Cow's milk is 15.91% (Nematollahi and Moghddam, 2008). On the other side, the observations made in the present study contrast the outcomes of a study made in China to determine the prevalence of the parasite's DNA in cow's milk, carried out in China, with a prevalence rate of 1.05% (Wang and Meng, 2020). The results of which do not agree with the Egyptian recording.

The results of the polymerase chain reaction technique confirmed that the prevalence of the parasite in buffalo milk was 0% (Fereig *et al*., 2022). A study was conducted in Iran to detect DNA from the parasite in unpasteurized buffalo milk and using molecular tests report parasite prevalence rate of 2.10% (Abdollahzadeh *et al*., 2022). The differences in the results may be due to several reasons, including the geographical location, weather conditions, and soil type, which are among the most important reasons for the high incidence of toxoplasmosis in camel milk in this region (Stelzer *et al*., 2019), as well as poor medical care and the migration of camels to semi-arid areas in search of a better range (Abdallah *et al*., 2020), essentially the age of the animal is another contributing factor as the older the animal, the more susceptible it is to toxoplasmosis (Gebremedhin *et al*., 2016). In addition to the possibility of camels coming into contact with small ruminants, as small ruminants are more susceptible to toxoplasmosis (Tilahun *et al*., 2018).

Several recent studies have shown that the prevalence of *Toxoplasma gondii* in milk is influenced by various environmental and management factors. For instance, studies conducted in Egypt found a significant correlation between the prevalence of *T. gondii* and the feeding practices of livestock (Saad *et al*., 2018). Additionally, geographic location plays a crucial role, with higher prevalence rates reported in humid tropical regions as compared to temperate and dry climates (Fereig *et al*., 2022). Furthermore, the susceptibility of camels to *Toxoplasma gondii* may be due to their exposure to contaminated environmental sources such as water and soil contaminated with oocysts shed by felines (Kazem, 2017). This highlights the need for stricter biosecurity measures in farms, especially in regions where camels are more frequently exposed to such environmental risks.

CONCLUSIONS

It can be concluded that *T. gondii* parasite DNA is found in the milk of cows, buffaloes, and camels. Health and veterinary departments must provide health guidelines to lactation breeders and milk label consumers every day in order to interfere with infection of animals and then transmission to human beings.

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NOVELTY STATEMENT

This study provides new insights into the prevalence of *Toxoplasma gondii* in milk samples from cows, buffaloes, and camels in Iraq. It also contributes to the understanding of zoonotic transmission risks from unpasteurized milk.

AUTHOR'S CONTRIBUTION

The principal investigator in study design, data harvesting and drafting of the manuscript was Israa Kareem Al-Zamili. Data interpretation and analysis was assisted by Hadi M. Al-Mayali and the manuscript undergone revisions by Hadi M. Al-Mayali to allow further development. The final manuscript version was approved by both authors and was made with both authors.

Ethical approval

The studies were conducted approved in the ethics code for use of animals. Approval of the Institutional Animal Care and Use Committee, with approval number (545), has been granted regarding ethical animal use by IACUC [Department of Biology, College of Education, University of Al-Qadisiyah] on 07/03/2025. Ethical principles were

followed on the welfare of all animals and nationally and internationally recommended treatment of animals were followed on animal use.

Consent for publication

The final version of the manuscript was subject to review by all authors and the approval by all authors for submission. If any given consents are needed, they have been received and there are no claims or restrictions with respect to the publication of this study.

Availability of data and material

The data sets used and examined in the study can be obtained ready for download by the corresponding author on satisfactory request. Interested researchers have ready access to supporting material or other raw data relevant to the outcomes of this study.

Generative AI and AI-assisted technology statement

The authors used Grammarly only to improve the language and readability of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflict of interest

The authors have declared no conflict of interest.

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