Short Reports Low Prevalence of Toxoplasma Gondii DNA in the Fresh Milk of Cattle in China

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Research article

Keywords: Toxoplasma gondii, Milk, Cattle, China

Posted Date: September 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-62998/v1>

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Abstract

Background: It is generally recognized that there is a risk for humans to acquire toxoplasmosis through consumption of raw milk from infected animals. Although several studies have been conducted to detect the seroprevalence of *T. gondii* in cattle in China, no study has been conducted to detect *T. gondii* DNA in milk of cattle in China. Thus, the present study was firstly conducted to explore the prevalence of *T. gondii* DNA in fresh milk of cattle in China.

Results: A total of 2092 blood samples and fresh bovine milk were collected from six provinces between January 2018 and June 2019, respectively, and examined by ELISA and semi-nested PCR, respectively. In total, 123/2092 (5.88%) of the blood samples and 22/2092 (1.05%) of the milk samples were positive for *T. gondii*, respectively. Fifteen of these 22 positive milk samples (68.18%) were animals who also recorded serologically positive. Moreover, cattle and milk from family farming have significantly higher *T. gondii* prevalence than those from commercial farm.

Conclusions: To our knowledge, this is the first report of *T. gondii* DNA was found in the fresh milk of cattle in China, suggesting that the consumption of raw milk from seropositive cattle could be a potential source of human infection.

Background

Toxoplasmosis is a global zoonotic parasitic diseases, caused by *Toxoplasma gondii*, an important opportunistic protozoa, which can cause great financial losses, especially for the animal production industries, and is of great importance for public health [1-3]. All kinds of hosts may be infected through ingestion of *T. gondii* oocysts or cysts containing bradyzoites, via ingestion of tachyzoites in non-pasteurized milk products or through the transplacental transmission [4].

Tachyzoites of *T. gondii* have been detected and isolated from the milk of numerous species and milk has been considered as a significant source of *T. gondii* infection in humans [4-8]. Experimental studies on animal models have indicated that tachyzoites existed in the milk of infected animals and could transmit to their offspring [5]. Moreover, some studies has shown that humans can be infected with *T. gondii* through consuming non-pasteurized goat milk [9,10]. Several studies have been conducted to detect the seroprevalence of *T. gondii* in cattle in China [11-15], but, until now, no study has been conducted to detect *T. gondii* DNA in milk of cattle in China. Thus, in view of this information is scarce, the present study was firstly carried out to explore the prevalence of *T. gondii* DNA in fresh milk of cattle in China.

Methods

Sample collection

A total of 2092 blood samples and fresh bovine milk were collected from six provinces (Heilongjiang, Jilin, Shandong, Hebei, Shanxi, Inner Mongolia and Xinjiang) (Figure 1) between January 2018 and June 2019, respectively. Cattle were handled with good ethical animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China. Permission has been obtained from the owners of cattle to collect the serum samples. In the present study, clinically healthy animals were sampled and sick animals were excluded. For blood sampling, 10 ml blood sampling of each cattle were collected from the tail vessel by sterile tubes sans/without anticoagulant. The blood samples were transferred to the lab by the ice box and stored at 4°C for 2 hour and then centrifuged at 1500×*g* for 10 min. After this, the serum was obtained and stored at -20°C for serology. For the milk sampling, teats was firstly been cleaned and disinfected with iodine solution (0.5%), then milk was manually collected. The first few squirts were excluded. Then, approximately 5 ml of milk was collected in sterile glass tubes and frozen for molecular examination.

Serology

IgG antibodies against *T. gondii* was detected by a commercially available ELISA kit (ID Screen® Toxoplasmosis Indirect, Montpellier, France) following the instructions of the manufacturer.

Molecular examination to detect the *T. gondii* DNA

The commercial QIAamp DNA mini kit (Qiagen, GmbH, Hilden, Germany) was employed to extract the genomic DNA from milk samples following the instructions of the manufacturer. After extraction, the DNA samples were stored at -20°C until used.

To detect the presence of *T. gondii* DNA in fresh milk of cattle, a semi-nested PCR targeting the *T. gondii* B1 gene was employed as previously described elsewhere [16]. Positive controls of DNA from parasite-infected mice and negative control from non-infected mice (PBS) were included throughout the whole examination.

Results

In the serum samples, 123/2092 (5.88%) of the animals were positive for *T. gondii* (Table 1). Among the age category, the highest seroprevalence was detected in cattle of 2-3 year old category (6.26%), followed by > 5 year old category (5.95%) and 4-5 year old category (5.63%). In view of the geographical origin, *T. gondii* seroprevalence varied in cattle from different regions, ranging from 4.14% in Shandong province to 6.59% in Inner Mongolia Autonomous Region (Table 1). Moreover, cattle from family farming (9.48%) have a significantly higher *T. gondii* seroprevalence than those from commercial farm (5.26%) ($P<0.01$). The samples collected in 2018 (5.78%) have a similar seroprevalence with those collected in 2019 (5.97%).

In the milk samples, the *T. gondii* DNA was detected in 22/2092 (1.05%) of the milk samples. Fifteen of these 22 positive milk samples (68.18%) were animals who also recorded positive samples in the

serology. The *T. gondii* DNA prevalence in >5 year old category (0.05%) was lower than that in other categories. Moreover, milk from family farming (3.27%) have a significantly higher *T. gondii* prevalence than those from commercial farm (0.07%) ($P<0.01$).

Discussion

It is believed that almost a third of human worldwide has been infected with *T. gondii* [2,17]. Regions with hot, humid climates and lower altitudes are considered to contribute to the infection of *T. gondii* [1]. Although *T. gondii* can infect all kinds of warm-blooded animal, cattle with a more effective immune response to *T. gondii* infection are considered as poor hosts for *T. gondii* compared with other animals, such as sheep or goat [18-20]. However, cattle tissues and unpasteurized milk has been proved to contain virulent *T. gondii* [20], suggesting that human may get infection through consuming the meat and milk.

In the present study, *T. gondii* DNA was detected in 22/2092 (1.05%) fresh milk samples of cattle, suggesting that natural infection has been occurred in these farms. This information is very important because some families in those farms consume milk very day, indicating that they are at a risk of exposure for *T. gondii*. Moreover, cattle and milk from family farming have significantly higher *T. gondii* prevalence than those from commercial farm, suggesting that cattle and milk from family farming have more chance to connect with *T. gondii*, thus, more preventive measure should be recommended in family farming. More disease awareness efforts should be carried out among individual farmers.

It is generally believed that tachyzoites, the stage most likely present in milk, could be immediately destroyed by the gastric juice when we consumed the unpasteurized milk or its products [21]. To our disappointment, ingested tachyzoites have been verified to can cause infection [22]. *T. gondii* exists in unpasteurized or unseemly processed milk or fresh cheese, can be a significant risk for the people lived in the rural regions because they are their major food source [23].

Positive results of the PCR showed that *T. gondii* DNA exist in the milk, but this result is still not enough to verdict the possible transmission of toxoplasmosis by directly ingesting the fresh milk because no living parasites have been isolated from the fresh milk in the present study. However, the present results have already indicated that chronic infection of *T. gondii* has been occurring in these positive cattle, especially for those of *T. gondii* DNA detection in milk who also recorded positive samples in the serology, providing useful information for future studies.

Conclusion

Although low prevalence of *T. gondii* DNA has been detected in the fresh milk of cattle by molecular analysis, it is still possible that toxoplasmosis transmission may occur in some rural regions because milk is used for subsistence without pasteurization or boiling prior to consumption. However, more studies should be carried out to explore the possible transmission of toxoplasmosis by directly ingesting the fresh milk or its products.

Abbreviations

ELISA: Enzyme-linked Immunosorbent Assay; PCR: Polymerase Chain Reaction

Declarations

Acknowledgements

The authors would like to thank the owners of cattle for their involvement in the study. We are grateful to the veterinary staff for their help with the collection of blood samples.

Authors' contributions

QFM conceived and designed the study and critically revised the manuscript. YLW collected the samples, performed the experiments, analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

Funding

Project support was provided by the Science and Technology Development Plan of Jilin Province (Grant No. 20180520034JH). The founder had no role in the study design, sample collection, detection, sequencing, analysis and interpretation, or the manuscript preparation.

Availability of data and materials

The datasets used and analyzed during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Animal Ethics Committee of Jilin Agricultural University (Approval No. JLAU 2018-006). The samples were collected and handled in accordance with the good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Seroprevalence of *T. gondii* infection in blood samples of cattle in China

Variable	Category	No. tested	No. positive	Prevalence (%)	<i>P</i> -value
Age	2-3 year	607	38	6.26	0.870
	4-5 year	1065	60	5.63	
	>5 year	420	25	5.95	
Region	Heilongjiang	263	16	6.08	0.931
	Jilin	344	19	5.52	
	Inner Mongolia	440	29	6.59	
	Shandong	241	10	4.14	
	Xinjiang	279	17	6.09	
	Shanxi	281	17	6.05	
	Hebei	244	15	6.15	
Feeding model	Commercial farm	1786	94	5.26	0.004
	Family farming	306	29	9.48	
Sampling time	2018 year	969	56	5.78	0.856
	2019 year	1123	67	5.97	
Total		2092	123	5.88	

Table 2. Prevalence of *T. gondii* DNA in fresh milk of cattle in China. Prevalence of *T. gondii* DNA in fresh milk of cattle in China

Variable	Category	No. tested	No. positive	Prevalence (%)	<i>P</i> -value
Age	2-3 year	607	9	1.48	0.298
	4-5 year	1065	11	1.03	
	>5 year	420	2	0.05	
Region	Heilongjiang	263	3	1.14	0.992
	Jilin	344	3	0.09	
	Inner Mongolia	440	4	0.09	
	Shandong	241	2	0.08	
	Xinjiang	279	4	1.43	
	Shanxi	281	3	1.07	
	Hebei	244	3	1.22	
Feeding model	Commercial farm	1786	12	0.07	<0.001
	Family farming	306	10	3.27	
Sampling time	2018 year	969	10	1.03	0.941
	2019 year	1123	12	1.07	
Total		2092	22	1.05	

Figures

Figure 1

The map of China, showing the locations of samples collected from cattle in the present study. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.