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Detection of *Toxoplasma gondii*-specific immunoglobulin (IgG) antibodies in meat juice of beef

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Article information	Abstract
<i>Article history:</i> Received March 21, 2020 Accepted April 22, 2020 Available online March 15, 2021	Toxoplasmosis is an important worldwide foodborne zoonotic disease. Infected cattle meats are considered a serous cause of human toxoplasmosis. Here, this study assesses the infection with <i>Toxoplasma gonddi</i> (<i>T. gondii</i>) in cattle using meat juice samples from diaphragmatic muscles collected at the slaughter. An in house indirect enzyme linked
<i>Keywords</i> : <i>Toxoplasma gondii</i> Beef Meat juice ELISA LAT	immunosorbent assay (ELISA), and commercial latex agglutination test (LAT) followed by immunoblotting were developed on the meat juice (fluids) using tachyzoites of locally isolated <i>T. gondii</i> strain. The comparative analysis of the results of the tested juice samples showed an excellent agreement between the in-house indirect ELISA and LAT test in the positive and negative of meat juice. Relative sensitivity was higher for ELISA on
<i>Correspondence:</i> R.M. Shaapan <u>rmshaapan2005@yahoo.com</u>	diaphragms fluids random samples 80.39%, for the LAT test was 68.6%. Immune-reactive bands of <i>T. gondii</i> local strain Ag with naturally infected meat juice were 116, 83, 65, 30 and 23 KDa. The obtained results concluded that the development of an effective ELISA test to be used in for detection of toxoplasmosis infection of slaughtered cattle in large-scale would be exactly valuable, since the important role that beef plays in epidemiology of <i>T. gondii</i> , in particular the hazard of transmission to human and food safety.

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Introduction

Toxoplasmosis is a world-wide distributed infection is due the infection by the zoonotic *Toxoplasma gondii* (*T. gondii*)) protozoal parasite which can be transmitted to humans and other animals; including cattle and it is an important foodborne zoonosis (1,2). Human and most of warm-blooded mammals become infected through ingestion of environmentally resist sporulated *T. gondii* oocysts from final host (cat) or ingest the infected intermediate host tissues containing *T. gondii* tissue cysts (3). The diagnosis of *T. gondii* infection in livestock animals and its originated food products is obstructed by the deficient of well effective procedures that can investigate viable *T. gondii* parasite infective stages in meat and tissues that make food dangerous for consumers (4).

Previously, studies in our laboratory used purified fractions to minimize or avoid the cross reactivity and increasing the sensitivity of available techniques to detect low levels of antibodies of toxoplasmosis in cattle and camel sera (5). Also, to diagnosis of ovine toxoplasmosis isolated cross reactive fraction was used as antigen in ELISA assay (6). Moreover, the isolation fraction from Fasciola worm at molecular weight 65 kDa used as diagnostic potency of toxoplasmosis in experimentally infected sheep serum using immunoblot method (7). Different degrees of specificity were found in sheep naturally and or experimentally infected when comparing ELISA dependent on crude or recombinant antigens (8) Whereas Mikaee et al. (9) used serological (ELISA) and molecular tools (PCR), to diagnose toxoplasmosis in sheep, showed that the PCR results were essential in the diagnosis of the infected animals more than ELISA techniques. Although Dong et al. (10) showed that

the detection of antibodies against T. gondii in dairy cattle was low prevalence. In Egypt, the antibodies against T. gondii prevalence in goat and sheep was high and may refers to the important role of mutton and other meat as transmissible media of human toxoplasmosis due to the habit of eating undercooked grilled meat (11). Recently, some alternative methods, based on antibody found in meat juice and multiple diagnostic assays are applied for serological investigation for toxoplasmosis, which is commonly made for the meat of different food animals (12). Fluids from muscle (meat juice) give an interesting alternative matrix because it can be easily collected than blood drawing and serum preparation (13). Several studies that have been done to examine antibodies against. T. gondii infection on meat juice, in domestic animals; pigs (14), rabbit (15), meat from wild boars (16), chickens (17), sheep (18). While livestock in particular cattle are one of the major meat sources in the world, people who eat infected T. gondii undercooked meat or raw cow milk can be expose to the infection (19).

In spite of the wide range of serologically available diagnostic tests routinely applied to investigate toxoplasma infection in animal groups of different species (20), there are no documented standard serological tests available at this time that accurate the relationship between sero-positivity and the parasitic infective stages existence in animal tissues and meat (21). The accuracy of diagnosis of varies methods of serological assays is commonly evaluated by comparing its findings with those of biological tests like bioassays in cats or mice as a standard gold test (22), or another different serological investigation with the high sensitivity and good specificity expected tests such as the Sabin Feldman (dye test), ELISA and modified agglutination test (MAT) (23). The using of different crude, fractionated, or recombinant antigen in ELISA procedures have been has been adapted d to find out the T. gondii antibodies in meat juice (18,24).

Due to suitable muscle fluid production from meat pieces and may be automated as a large-scale analysis, also offer the opportunity for parasitological evidence. The aim of this study was to estimate the potential of cattle meat juice infection with *T. gondii* serologically, using indirect home ELISA and compared to commercial LAT, to assess the feasibility of such a strategy to control toxoplasmosis.

Materials and methods

Meat samples

Diaphragmatic meat samples of 51 cattle carcasses were collected in Cairo slaughterhouses unidentified age. Diaphragms samples were individual cut into small pieces (about 25 gm) and kept in plastic bags at -20°C until examined (25).

Meat juice (fluid) collection

Meat juice from each frozen diaphragm sample were obtained individually, thawing at room temperature, then fluids were collected with a pipette into a small tubes and labeled, as the previously described technique by Nockler *et al.* (13).

Preparation of T. gondii tachyzoites antigen

T. gondii infective stages had been isolated from the pooled meat sample (heart and diaphragm) obtained from slaughtered sheep as the method carried out by Shaapan and Ghazy (26). Virulent strain of *T. gondii* was obtained by feeding of pooled meat samples of sheep to cats according of Davis and Dubey (27) method and then isolated parasite maintained in our laboratory through the serial passage in mice peritoneum. *T. gondii* tachyzoites antigen was prepared and assayed for protein content (28).

Enzyme Linked Immunosorbent Assay (ELISA)

Indirect ELISA was adopted to detect the *T. gondii* IgG in meat juice toxoplasmosis infected diaphragms, using tachyzoites antigens of virulent local strain of *T. gondii*. The assay was conducted as described by method of Shaapan *et al.* (29) with some modifications. Optical density was recorded at 405 nm with automated ELISA reader (BIOTEC, LX800, USA). The cutoff was determined as the two times of the standard deviation plus mean of negative fluid and the optimal concentration of antigen, antibody and conjugate were estimated after the checker- board titrations (30).

Toxoplasma IgG Latex agglutination test (LAT)

Commercial LAT (Nova Test), is one step Diagnostic Rapid Test, depending on the Gold Immuno-Chromatography Assay (BEIJING) was used for anti *T. gondii* IgG detection in diaphragms fluids samples.

SDS Electrophoresis

Protein components of the prepared virulent local strain of *T. gondii* electrophoretically separated on 10% slab SDS-PAGE according to the procedures mentioned by Laemmli (31). The separated gel was fixed in 50% methyl alcohol, stained with silver nitrate (32).

Immunoblot

Proteins of *T. gondii* antigen was fractionated by SDS-PAGE transferred to nitrocellulose membrane (33). After washing and blocking, apply the incubation of the membrane with infected meat juice diluted at 1:10. Whereas, antibovine IgG horse radish peroxidase-conjugate (sigma, USA) was used at dilution 1:1000.

Results

T. gondii IgG in meat juice using ELISA

ELISA was performed to evaluate the success of tachyzoites of local strain of *T. gondii* antigen to detected toxoplasmosis antibodies IgG in diaphragms meat juice. Sensitivity was 80.39% (Figure 1).

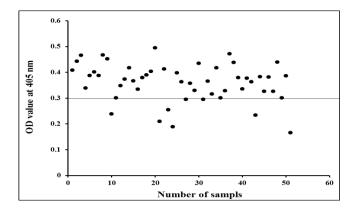


Figure 1: Diagnostic potential of tachyzoites of local *T*. *gondii* strain antigen in diaphragms meat juice toxoplasmosis

T. gondii IgG in meat juice using LAT

Toxoplasmosis antibodies IgG detected by recombinant *Toxoplasma* antigen in Colloidal Gold Chromatography (Cassette), commercial Rapid test (LAT). Positive and negative fluid (meat juice) samples were observed (Figure 2).



Figure 2: Diagnosis of meat juice toxoplasmosis by commercial diagnostic LAT test (Cassette); two colored bands in positive meat juice sample (A) and one band in negative meat juice sample (B).

Comparing of ELISA with LAT

Out of 51 fluids diaphragms analyzed random samples 41(80.39%) and 35(68.6%) fluid samples were positive by local strain *T. gondii antigen* using ELISA and LAT assays respectively. Of 35 positive meat fluid samples with LAT 31(60.79%) were positive by local strain *T. gondii antigen* ELISA (Table 1).

T. gondii antigen SDS

Electrophoretic profile of *T. gondii* tachyzoites Ag showed 10 bands were detected at 165, 116, 97, 83, 76, 65, 53, 30, 27and 23 kDa (Figure -1 Lane A) (Figure 3).

Table 1: Immunodiagnosis of toxoplasmosis in diaphragms meat juice by ELISA compared with commercial LAT

LAT	ELISA		Total
	+	-	Total
+	31 (60.79%)	4 (7.84%)	35 (68.63%)
-	10 (19.61%)	6 (11.76%)	16 (31.37%)
Total	41(80.39%)	10 (19.61%)	51(100%)

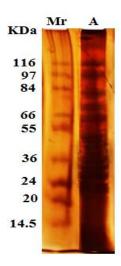


Figure 3: electrophoretic profiles of *T. gondii* local strain Ag (Lane A). Molecular weight standards (Lane Mr.)

Immunogenic Bands

Identified immune-reactive bands of *T. gondii* local strain Ag with naturally infected meat juice were 116, 83, 65, 30 and 23 KDa (Figure 4).

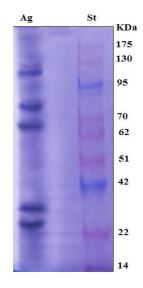


Figure 4: Immune-reactive bands identified by positive meat juice against local of *T. gondii* strain (Lane: Ag). Standard molecular weight (lane: St).

Discussion

In this study the in-house indirect ELISA using tachyzoites antigen of local isolated *T. gondii* is effective and specific test compared with a commercial LAT as the main standard were used for *T. gondii* antibodies standard in cattle meat. These tests have been described elsewhere to detect infection in meat and tissues that are commonly diagnosed by examining other zoonotic diseases such as trichinella in experimentally infected porcine tissues (13), and has been widely accepted, suitable, sensitive and may be used confidentially for screening animals against toxoplasmosis regardless of the host species (34).

The detection anti-Toxoplasma IgG antibodies in samples of tested meat juice obtained from 51 previously infected cattle diaphragm tissues revealed high sensitivity 80.39% using ELISA than the commercial LAT Rapid kit 68.63%. Similar values of results 87.4% in sheep have been documented by Verhelst et al (35). In addition, these results agreement with Hill et al. (36), when reported the sensitivity values for tissue fluid ELISA was 79.9% in swine and was lower values using PCR method. Moreover, Ahmed et al. (37) revealed that the prevalence of infection among the examine camels blood samples in Egypt by indirect ELISA was 52.5% and by using PCR was 24%. In addition, this high sensitivity of meat juice ELISA than the commercial LAT rapid test for determining infection was reported by other authors before (38,39). Moreover, Vismarra et al. (18) showed that the T. gondii identification in sheep using molecular investigation in tissue or meat samples was less sensitive 45.4% than serological one 100% for assess the infection status. Various previous researches have reported that the infection rats of T. gondii in small ruminants range from 66% to 97% (40-42).

The prevalence of human toxoplasmosis associated with foodborne infection was about 50%, corresponding to raw or undercooked meat consumption (43). In addition to that the local cattle meat are riskier than the imported due to the higher rate of infection with *T. gondii*, the parasite can infect all examined organs (heart, muscles, tongue), and the infection rate effected by season not by age of animals (44). Moreover, Mikaeel and Al-Saeed (45) found that high infection rate of *T. gondii* in free range local chickens in Duhok Province and chickens of older age showed higher infection rates than younger chickens, this due to the number of times to exposure which increased with age. So it must be follow strict hygienic measures in order to minimize the transmission of infection to human.

During slaughter inspection, there is no method to differentiate the infected *T. gondii* from the uninfected animal carcasses and the parasite tissue cysts remain viable for the lifetime of the animal; thus, all edible parts were seropositive for *T. gondii* (46). Our results devised a possible alternative protocol through using diaphragm meat juice for determination toxoplasmosis in cattle without need for

experimental infection and the bioassay of parasite in laboratory animals to confirm the a live or viable parasite stages of infection presence and without needing to additional equipment other than that required for ELISA and also this study explain the comparative results of ELISA with that of commercial LAT. Furthermore, the immunoblotting proved immune-reactivity of samples of meat juice. In fact, sensitivity of toxoplasmosis in meat juice affected by the amount or concentration of blood in the tissue and not a local detection of the specific antibodies (24). Looking at the overall ELISA results on tissue and meat samples can be useful to conduct a serological and epidemiological survey. It may give an acceptable sensitivity in relation to the simplicity of use (4).

Conclusion

The study results provide a basic information on the toxoplasmosis in cattle meat juice and indicate the significant human health and hygiene risks corresponding with consuming raw and undercooked beef. The indirect ELISA was a simple, rapid, inexpensive and more sensitive for diagnosis *T. gondii* in meat juice and it can be considered as a promising test for monitoring Toxoplasmosis in meat and meat products of cattle. The meat juice (fluid) was helpful as an acceptable sample for detecting antibodies to *T. gondii* using ELISA and diaphragmatic tissues are a potential matrix for parallel seropositive diagnosis.

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Conflict of interest

The authors of the current work declare that they have no conflicts of interest in this work.

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الكشف عن الأجسام المضادة للجلوبيولين المناعي النوعي ج لطفيل المقوسة الكوندية في عصير لحوم الأبقار

رأفت محمد شعبان'، نجوى إبراهيم تعيلب' و إيمان حسبن عبد الرحمن

اقسم الأمراض المشتركة، أقسم الطفيليات وأمراض الحيوان، شعبة البحوث البيطرية، المركز القومي للبحوث، الدقي، الجيزة، مصر

الخلاصة

المقوسة الكوندية هو طفيل اولى ينتقل عن طريق الأغذية وينتشر في جميع أنحاء العالم. وتعتبر لحوم الأبقار المصابة به سببًا لمرض خطيرًا هو داء المقوسات الغندية الذي ينتقل للبشر. وفي هذه الدراسة تم تقيم معدل انتشار الإصابة بالمقوسات الكوندية في الأبقار باستخدام عينات عصير اللحم من عضلات الحجاب الحاجز التي تم جمعها عند ذبح هذه الأبقار. تم تطوير اختبار الممتص المناعي المرتبط بالإنزيم غير المباشر المجهز محليا واختبار تراص اللاتكس التجاري متبوعاً باختبار الطبع المناعى على عصير اللحوم (السوائل) باستخدام مستضدات محضره من الأطوار المعدية للطفيل من سلالة المقوسة الكوندية المعزولة محلياً. وقد أظهر التحليل المقارن لنتائج اختبار عينات عصير اللحوم توافقًا ممتازًا بين اختبار الممتص المناعي المرتبط بالإنزيم غير المباشر واختبار تراص اللاتكس التجاري في الإيجابية والسلبية لعصير اللحوم. وكانت الحساسية النسبية أعلى في اختبار الممتص المناعي المرتبط بالإنزيم غير المباشر في عينات عشوائية من سوائل الأنسجة اللحوم المختبرة ٣٩, ٨٠٪، ولكن اختبار تراص اللاتكس التجاري أعطى حساسيه أقل ٦٨,٦% وكانت الأوزان الجزيئية للأشرطة البروتينية المرتبطة والمتفاعلة مناعيا من المستضد المحضر من سلالة المقوسة الكوندية المعزولة محليًا مع عصير اللحم المصاب طبيعيا هي ١١٦ و ٨٣ و ٦٥ و ٣٠ و ٢٣ كيلو دالتون. وخلصت النتائج التي تم الحصول عليها إلى أن بتطوير اختبار اختبار الممتص المناعى المرتبط بالإنزيم غير المباشر يكون فعال وذو قيمة كبيرة لاستخدامه على نطاق واسع للكشف عن عدوى داء المقوسات الكوندية في عصير اللحوم للماشية المذبوحة، ونبهت الدراسة للدور الهام الذي يلعبه لحم البقر في وبائيات طفيل المقوسات الكوندية وعلى وجه الخصوص خطر انتقاله إلى الإنسان مع الحفاظ على سلامة الغذاء للمستهلك.