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Original Research Article

LATEX AGGLUTATION TEST AND PCR ASSAYS FOR DIAGNOSIS OF *Toxoplasma gondii* INFECTION IN RED MEAT PRODUCING ANIMALS IN ASWAN GOVERNORATE, SOUTHERN EGYPT

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Abstract: Livestock meat is considered a potential source of human infection, therefore evaluation of the infection rate with *Toxoplasma gondii* (*T. gondii*) in these animals' meat is needed for public health protection. Serum and meat samples were collected from slaughtered animals (n=106) (27 sheep, 42 cattle and 37 camels) from slaughterhouses in Aswan Governorate, located in southern Egypt. *T. gondii* infection status was detected by Latex agglutination test (LAT), microscopic examination and PCR. Our results revealed that the overall prevalence of anti-*T. gondii* antibodies among serum samples was 48.1 % using LAT. Male sheep, cattle, and camels had a higher positive percentage (42.9 %, 69.4% and 33.3 %, respectively) than females (40 %, 50 % and 30.8 %, respectively). The infection rate of *T. gondii* was higher in adult sheep and camels, while it was lower in adult cattle (64.5 %) than in young (72.7 %). There was a high statistically significant difference ($P \leq 0.01$) between the animal species using LAT. *T. gondii* trophozoites were detected microscopically in 47.1 % of meat samples, while *T. gondii* DNA was detected in 56.9 % by using conventional Polymerase Chain Reaction (cPCR) based B1 gene. There was no significant difference between the results of both microscopy and PCR methods. In conclusion, PCR outperforms LAT in the detection of *T. gondii* infection and can thus be applied to routine diagnosis of toxoplasmosis in red meat producing animals.

Key words: Aswan; camel ; cattle ; Egypt; LAT; PCR; red meat; sheep; *Toxoplasma gondii*

Introduction

The zoonotic protozoan parasite, *Toxoplasma gondii*, has a worldwide distribution and global significance. It infects all warm-blooded vertebrates including mammals, birds, and humans (1-4). Toxoplasmosis is a primary concern among the five parasitic diseases targeted by public health initiatives since it affects one-third of the world's population. It causes congenital disease and abortion in both humans and livestock leading to substantial economic losses in these hosts, hence gaining great clinical and veterinary importance (5, 6). Animals are of zoonotic impact and are regarded as highly prevalent sources of human toxoplasmosis via their meat containing viable *T. gondii* tissue cysts (7). Humans may acquire infection through ingestion of tissue cysts in undercooked or raw meat; ingestion of food and water contaminated with oocysts shed in cat's feces after being sporulated or congenitally infected through trans-placental transmission of tachyzoites (8).

Toxoplasma gondii in animals can be detected either by finding the protozoon's antibodies serologically, its developmental stages microscopically, or its nucleic acid by molecular methods (9). Serological assays such as Latex agglutination test (LAT) are rapid and have good accuracy for detecting anti-*T. gondii* antibodies, but they don't provide a good indication about presence of infectious tissue cysts. Although theoretically, there is a strong correlation, as both antibodies and tissue

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cysts are assumed to persist life-long, studies comparing indirect and direct detection methods are limited (10-12). Toxoplasma DNA in meat samples of seronegative animals has been demonstrated using PCR (13, 14). Several different targets are available for PCR-based detection of *T. gondii*. B1-gene and the 529 base pair (bp) repeat element are the most common targets (15).

To the authors' knowledge, the evaluation of the PCR test for detecting the protozoan-parasite DNA from animal tissues is not yet conducted in the study area. Therefore, the objectives of the present study were to (i) determine the infection rate of *T. gondii*, in different animal species with regard to sex, and age as risk factors, among red meat-producing animals using LAT; and (ii) to use microscopy and PCR assays for confirmation of the *T. gondii* infection in red meat tissues.

Materials and methods

Study area

The present research was carried out in Aswan, the southern region of Egypt, where 60% of the population lives in rural areas. The study area has a hot desert climate and is located at a latitude of 24° 5' 20.18" N and a longitude of 32° 53' 59.39" E. The study was conducted on red meat animals including sheep, cattle, and camels at the central slaughterhouses from January to August 2017. Data on animal species, sex, and age were recorded.

Serum separation and LAT examination

A total of 106 blood samples were collected from slaughtered animals (27 sheep, 42 cattle, and 37 camels) via jugular vein puncture and were used for serum separation. All sera were tested for the presence of specific antibodies against *T. gondii* using Atlas Toxo latex kit (Atlas Medical, William James House, Cowly Rd., Cambridge, CB4 oWX, U.k.) (16).

Tissue samples

Heart, liver, and diaphragm specimens were obtained from the examined 106 slaughtered animals (17). About 100 gm of the tissues were aseptically taken in sterile plastic bags and then transferred in an ice box to the laboratory at the Department of Parasitology, Faculty of Veterinary Medicine, Assiut University. The three organs of each animal were minced together as one sample. The minced samples were divided in sterile labeled plastic bags into two parts, the first of which about 50 gm were stored at -20°C and used for microscopic examination, and the second part (50 gm) was stored at -80°C for further molecular examination.

Microscopic detection of Toxoplsma gondii

From the seropositive animals, meat samples were chemically digested (18) and the sediments were stained with Giemsa stain (19) then examined under a 100x objective oil immersion microscope for the presence of *T. gondii* trophozoites or cysts.

Molecular diagnosis

The minced samples (heart, liver, and diaphragm) of the seropositive animals (11 sheep, 28 cattle and 12 camels) were confirmed by the detection of the genomic DNA of *T. gondii* using cPCR targeting B1 gene (20), the PCR was carried out at the Molecular Biology Unit, Assiut University using the specific primer pair (B22-B23), since it is a 35-fold repetitive gene with 2214 nucleotides in each repeat (21). The sequence of forward and reverse PCR primers was (5'AACGG GCGAGTAGCACCTGAGGAGA3'and 5'TG GGTCTACGTCGATGGCATGACAAC3'), which amplify 115-bp sequence. The oligonucleotide primers used for the PCR were obtained from Invitrogen ™, Merelbeke, UK. DNA was extracted using Qiagen extraction tissue kit (QIAamp ® DNA Minikit, Hilden Germany) following the manufacturer's guidelines. The reaction conditions were one initial denaturation cycle for 5 min at 95° C followed by 45 cycles of denaturation step at 95° C for 40 sec, followed by annealing at 62° C for 40 sec. and extension at 72° C for 40 sec. Finally, the procedure was completed by over extension cycle for 10 min. Each amplification run contained two controls; DNAase free water as a negative control and the RH strain as a positive control isolated from a horse brought from Faculty of Veterinary Medicine, Cairo University and maintained in mice at the laboratory of Parasitology Department, Faculty of Medicine, Assiut University. For visualization of the amplified products, 10 µL of each PCR product were electrophoresed in the previously prepared agarose gel in horizontal gel electrophoresis. The images were analyzed by Gel Imager and Documentation System (Compact M, Biometra, Germany) (22).

Table 1: Sero-prevalence of *T. gondii* among sheep, cattle, and camels by LAT

Examined animals	Total serum samples	Positive samples (%)	P value
Sheep	27	11 (40.7)	0.007**
Cattle	42	28 (66.7)	
Camels	37	12 (32.4)	
Total	106	51 (48.1)	

Chi-square test, (*) statistically significant difference ($p \leq 0.05$); (**) highly significant difference ($p \leq 0.01$)

Table 2: *T. gondii* infection rate by LAT concerning gender and age of the animals

Animal species	Male +ve (%)	Female +ve (%)	P value	Adult +ve (%)	Young +ve (%)	P value
Sheep	3/7 (42.9)	8/20 (40)	0.89	7/14 (50)	4/13 (30.8)	0.31
Cattle	25/36 (69.4)	3/6 (50)	0.35	20/31 (64.5)	8/11 (72.7)	0.50
Camels	8/24 (33.3)	4/13 (30.8)	0.87	5/10 (50)	7/27 (25.9)	0.16
Total	36/67 (53.7)	15/39 (38.5)	0.12	32/55 (58.2)	19/51 (37.3)	0.11

Chi-square test, (*) statistically significant difference ($p \leq 0.05$); (**) highly significant difference ($p \leq 0.01$).

Table 3: Detection of *T. gondii* among the red meat samples by microscopic examination

Animal species	Total Examined	Number of positive samples by microscopy (%)	
			P value
Sheep	11	9 (81.8)	0.43
Cattle	28	10 (35.7)	
Camels	12	5 (41.7)	
Total	51	24 (47.1)	

Chi-square test, (*) statistically significant difference ($p \leq 0.05$); (**) highly significant difference ($p \leq 0.01$)

Table 4: Detection of *T. gondii* among sheep, cattle, and camels by molecular examination (PCR)

Animal species	Total examined No.	Positive samples by PCR		
		No. +ve	%	P value
Sheep	11	9	81.8	0.17
Cattle	28	14	50	
Camels	12	6	50	
Total	51	29	56.9	

Chi- square test, (*) statistically significant difference ($p \leq 0.05$) (**) highly significant difference ($p \leq 0.01$)

Table 5: Comparison between results of microscopy and PCR for detection of *T. gondii*

Animal species	Total examined No.	Detection methods				P value
		Microscopy		PCR		
		+ve	%	+ve	%	
Sheep	11	9	81.8	9	81.8	1.00
Cattle	28	10	35.7	14	50	0.28
Camel	12	5	41.7	6	50	0.68
Total	51	24	47.1	29	56.9	0.32

Chi- square test, (*) statistically significant difference ($p \leq 0.05$) (**) highly significant difference ($p \leq 0.01$)

Statistical analysis

The data are described by number and percent of positive cases (N, %). The Pearson Chi-square test was used to compare it. P value ≤ 0.05 was considered statistically significant. All test results were performed with the IBM SPSS software version 20.0 (SPSS Inc, Chicago, IL, USA).

Ethical statement

The samples were collected in accordance with ethical animal guidelines and regulations set by the animal care committee of the Faculty of Veterinary Medicine, Assiut University, and were in accordance with the internationally accepted principles animal care and use. Ethical approval was granted by the Research and Ethics committee of the Faculty of Veterinary Medicine, Assiut University.

Results

In the current study, out of 106 examined serum samples from the animals under investigation, 51 (48.1%) were serologically positive for *T. gondii* antibodies using LAT. Cattle has the highest infection rate (66.7 %), meanwhile it was 40.7 % in sheep, and camels showed less susceptibility to *T. gondii* infection (32.4 %). There was a high statistically significant difference (P ≤ 0.01) between the examined animal species using LAT examination (Table 1). Positive reaction for anti-*T. gondii* antibodies was seen macroscopically as visible white agglutination on the specific slide of LAT immediately after removing it from the rotator (Figure 1).

Out of 106 serum samples, male animals showed high infection rates of *T. gondii* (53.7 %) more than females (38.5 %). Male of sheep, cattle, and camels were more susceptible to infection 42.9 %, 69.4 % and 33.3 % than females 40 %, 50 % and 30.8 %, respectively. There was no significant variance in animal gender between different species. Furthermore, the infection rate of *T. gondii* was higher in adult (58.2 %) than young animals (37.3 %). Adult sheep and camels represented the higher infection rate 50 % and 50 %, respectively, while the lower infection rate was recorded in young animals (30.8 % and 25.9 %), respectively. Adult cattle have lower infection rate (64.5 %) than young cattle (72.7 %). There was no significant difference between the two age groups among species as well as within the same species (Table 2), (Figure 2).

From all the sero-reactive animals, 47.1 % of the examined tissue samples were positive microscopically for *T. gondii*. Sheep represented the most susceptible species to *T. gondii* (81.8 %), followed by camels (41.7 %) and cattle (35.7 %) (Table 3), (Figure 3, 4).

Toxoplasma gondii genomic DNA in minced tissue samples of sero-reactive animals was detected by cPCR (Figure 5). PCR amplification of the highly specific B1 region of *T. gondii* showed amplification products with a single band at 115 bp. Out of 51 tissue samples, 56.9 % gave positive results. Sheep had the highest positivity (81.8 %), while cattle and camels showed a 50% positivity rate (Table 4). There was no significant difference between results of microscopy and PCR (Table 5).

Discussion

This study shed light on the *T. gondii* infection rate among the red meat producing-animals (sheep, cattle, and camels) in Aswan Governorate, southern Egypt and highlighted the significant public health risk in this area. The overall infection rate of *T. gondii* among the examined animals using LAT was 48.1 %. These results were nearly similar to those of Khalil and Elrayah (16) in Sudan, who recorded 38 % seropositivity of *T. gondii* among the same animals. The difference in *T. gondii* infection rates among animal species may be due to differences in susceptibility to infection, immunity, and breed.

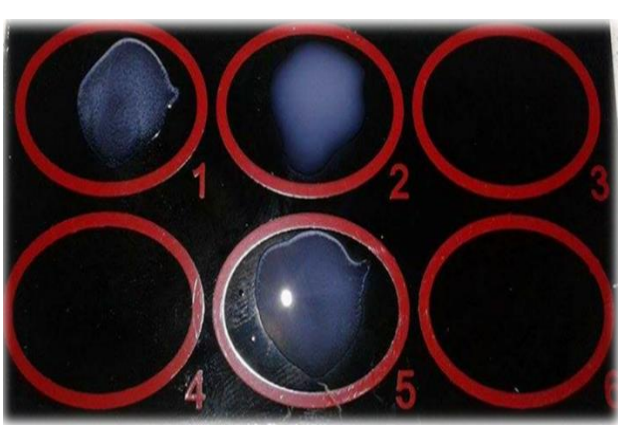

Figure 1: Toxo-Latex slide agglutination test showing *T. gondii* antigen-antibody reaction. Circle (1): A positive serum sample showing the white agglutinate. Circle (2) represents negative control, while Circle (5) represents positive control

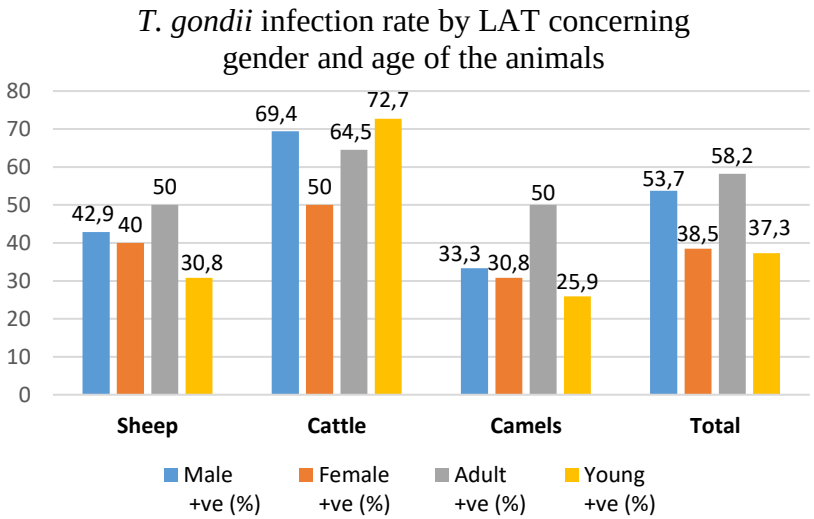

Figure 2: *T. gondii* infection rate by LAT concerning gender and age of the animals.

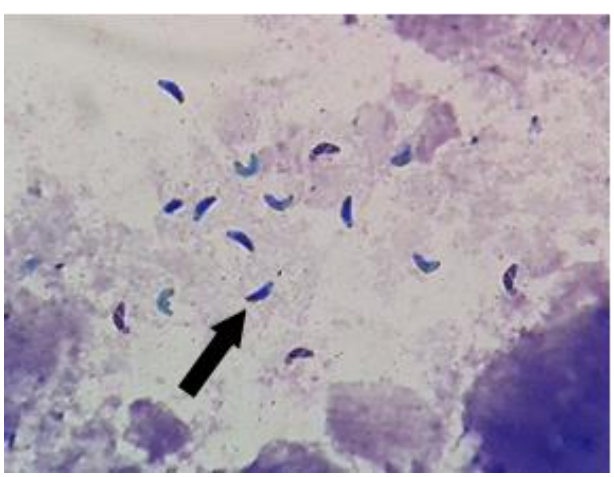

Figure 3: Geimsa-stained smear of tissue sediment, prepared from meat of sheep showing banana shaped *T. gondii* trophozoites (arrow) by (x100)

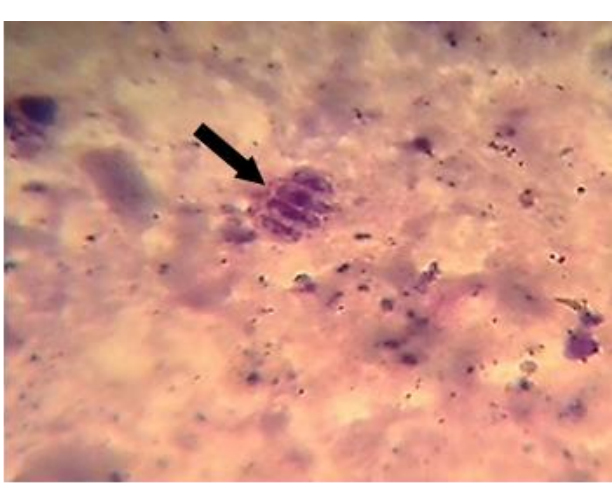

Figure 4: Geimsa-stained smear of tissue sediment prepared from meat of camel showing *T. gondii* trophozoites (arrow) by (x100)

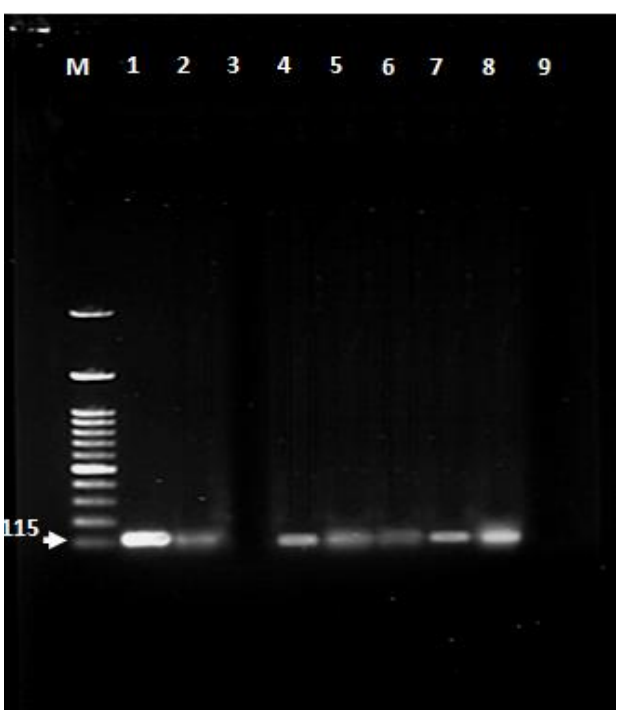

Figure 5. Agarose gel electrophoresis 1.5% stained with ethidium bromide showing PCR products of B1 gene illuminate (115 bp) specific for *T. gondii* detected in different tissue samples of sheep. Lane M: 100 bp ladder as molecular size DNA marker. Lane 1: Control positive to *T. gondii*. Lanes 2, 4, 5, 6, 7, and 8: Positive sheep samples. Lane 3: Negative sheep sample. Lane 9: Negative control.

The current study reported that 40.7 % of the examined serum samples from sheep were positive for *T. gondii*, which is nearly similar to previous reports recorded 41 % and 34.5% in Giza, Egypt (23) and Somalia (24), respectively. Lower infection rates were recorded for instance; 26.1% in sheep in some African countries (10), 21.5% in mutton meat in Iraq (25), and 23.4% in Saudi Arabia (26). A higher result was recorded in Sudan (57.5%) (16), and Ethiopia (58.8%) (27) by LAT. The variations in infection rates in sheep can be explained by different sampling strategies as some study's samples were from areas of suspected disease occurrence, which have flocks with a history of *T. gondii* abortions (28). Sheep is free-graze livestock and are constantly in contact with environments contaminated by *T. gondii* oocysts, making them more susceptible to infection (23). Another explanation may be that in villages, animals do not eat garbage and are usually accompanied by guard dogs, which prevent the entrance of cats (definitive host) to animals and reduce environmental contamination with Toxoplasma oocysts shed in cat's feces (29).

In the present study, we recorded an infection rate of 66.7 % with *T. gondii* among the examined cattle by LAT. The prevalence rate of *T. gondii* was higher than 29.4% reported in Egypt (Giza) (30), 32% in Sudan (16), 15.2% in Iraq (25), 13% in Poland (31) and 7.1% in Somalia (24), respectively. Tonouhewa et al. (10) observed a significant variation in the seroepidemiological data in cattle depending on four databases from different African countries and gave an overall estimated prevalence of 12 %. In the current study, nearly, most cattle slaughtered in Aswan abattoirs were imported from grazing herds in Sudan. Schoonman et al. (32) mentioned that grazing herds, as opposed to zero-grazed animals, have a greater chance of exposure to sporulated oocysts. Notable variations in the infection rates can be justified by differences in the management of the farms, levels of natural immunity, and sensitivity and specificity of the diagnostic test used. Moreover, high prevalence of toxoplasmosis of cattle in some areas may be attributed to humid and temperate climate; the absence of routine treatment against feline toxoplasmosis; exposure to cats and their oocysts also, larger herds have an increased chance of infection (10). In the present study, the sero-prevalence of *T. gondii* among camel samples was 32.4 %. Our findings agree with those previously reported in Egypt (33), different African countries (10) and Iraq (34) with infection rates 41.2 %, 36 % and 25.2%, respectively. This agreement might be explained by similar environmental conditions. Our results were higher than the results of Khalil and Elrayah (16) in Sudan, Kadly (24) in Somalia and Al-Anazi (35) in Saudi Arabia as they recorded 20 %, 6.3 % and 13.1 % prevalence rates with *T. gondii*, respectively. The difference in infection rates may be due to different tests used (16). Camels are kept most of the time in the desert, as there is limited contact with definitive hosts (cats); therefore, *T. gondii* infection in camels was low. Remarkably, higher prevalence rates than our results were reported in Central Sudan (44%) (36), and in Eastern Sudan (37) (49.7%). The difference between the present study and the other reports might be due to the different types of camel management systems and the number of samples taken using different serological techniques (35). The knowledge of potential associated factors with the infection of farm animals with the parasite is of fundamental importance to reduce the risk of human infection with *T. gondii* and implement the Hazard Analysis and Critical Control Points (HACCP), allowing the farmers to develop efficient and sustainable control measures against *T. gondii* infection in their farms (15). In this study, it was found that infection rate of *T. gondii* was higher in male animals (53.7%) than in females (38.5%), using LAT indicating high immunity in females, which may be due to the presence of estrogen, which normally increases immunity, whereas androgen in males decreases immunity. Regarding to age, a higher infection rate was found in adult animals (58.2 %) than in young (37.3 %). Our results concerning the LAT infection rate were nearly similar to previous reports from Egypt (38), Central Sudan (36) and Iraq (34). The high seropositivity observed in adult animals can be explained by excessive exposure to *T. gondii* oocysts during animal growth from the contaminated environment. They acquired the infection via ingestion of sporulated oocysts in soil and water contaminated by feline feces as previously documented (10, 27, 39).

In the current study, PCR was superior in *T. gondii* detection with 56.9 % than the microscopic examination (47.1 %). LAT requires further confirmation testing (40, 41). Generally, our results agreed with most studies, which signified sheep, cattle, and camels as the most *T. gondii* susceptible species. The equivalent digested tissue samples with positive *T. gondii* DNA reflect higher PCR sensitivity especially to B1 gene than microscopic ones (8, 14, 41). Furthermore, it should be noted that serological assays primarily detect chronic *T. gondii* infection in animals. Moreover, not all assays are suitable for every animal species and cross-reactions with antibodies to related parasites may result in false positives (42). Hence, PCR-based techniques have been developed to detect *T. gondii* DNA in tissue samples even in early infection (43).

Conclusion

In this study, the overall infection rate of *T. gondii* in red meat animals in Aswan Governorate, Egypt using LAT, was 48.1 %. The highest rate was found in cattle, followed by sheep and camels. Concerning the sex and age risk factors, anti-*T. gondii* antibody was higher in male animals than in females, adults have a higher rate of infection in sheep and camels. In contrast, in cattle, young animals were more susceptible. PCR outperforms LAT in the detection of *T. gondii* infection and can thus be applied to routine diagnosis of toxoplasmosis in red meat producing animals.

The authors declare that they have no competing interests.

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