agriculture

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Seroprevalence and Potential Risk Factors of *Toxoplasma gondii* in Dromedary Camels

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Abstract: (1) Background: *Toxoplasma gondii* (*T. gondii*) is one of the most prevalent parasites to affect humans and animals; (2) Methods: From January to December 2020, using a commercial enzyme-linked immunosorbent assay (ELISA) kit, a cross-sectional study was conducted to establish the seroprevalence of *T. gondii* in 390 dromedary camels raised in three governorates in Egypt and to identify the potential risk factors associated with infection; (3) Results: Overall, *T. gondii* seroprevalence in camels was 46.9%. Moreover, locality, sex, age, contact with small ruminants, history of abortion, and number of parities were found as risk factors for *T. gondii* infection in univariable analysis. The seropositivity to *T. gondii* increased significantly in camels living in Marsa Matrouh (OR = 2.02), among camels of more than 8 years old (OR = 5.28). Additionally, the likelihood of acquiring *T. gondii* infection was increased in camels that had contact with small ruminants (OR = 3.85) and a history of abortion (OR = 3.84) with these having parity more than four times (OR = 17.72); (4) Conclusions: The evaluation of seroprevalence and related risk factors for *T. gondii* infection is crucial for implementing an effective control programme to minimise and control *T. gondii* infection in camels and, as a result, transmission to humans.

Keywords: *Toxoplasma gondii*; ELISA; risk factors; dromedary camels; Egypt

Citation: Selim, A.; Marawan, M.A.; Abdelhady, A.; Wakid, M.H. Seroprevalence and Potential Risk Factors of *Toxoplasma gondii* in Dromedary Camels. *Agriculture* **2023**, *13*, 129. https://doi.org/10.3390/agriculture13010129

Academic Editor: Agustín Estrada-Peña

Received: 2 December 2022
Revised: 24 December 2022
Accepted: 2 January 2023
Published: 4 January 2023

1. Introduction

The camel is referred to as the "ship of the desert" because of its exceptional ability to survive in hot, arid conditions. It is also important economically to Egypt because it can be used as a source of milk and meat, a means of transport, for racing, and for tourists rides because the camel is associated with the history of the great Egyptian civilization. The problem of a red meat shortage in Egypt might be resolved by intensively feeding camel calves to increase their weight. Furthermore, due to its medicinal qualities, its flesh is a healthy choice and is high in protein and iron while being low in fat and cholesterol [1–4].

Camelids are often thought to have a low reproductive efficiency [5]. In nomadic herds, birth rates seldom reach 40%, whereas in more intensive herds, birth rates rarely exceed 70% [6]. Camels are afflicted with a variety of abortion-causing diseases, such as brucellosis, placentitis or uterine infections, toxoplasmosis, and trypanosomosis [7–10].

Camels were thought to be resistant to the majority of livestock diseases, but as more research studies were done, it was discovered that camels are vulnerable to a wide variety of pathogenic agents, including *Brucella* spp., *Toxoplasma gondii (T. gondii)*, *bluetongue* virus, *Mycobacterium avium* subsp. *paratuberculosis*, *Neospora caninum*, *Coxiella burnetii*, and *Staphylococci* spp. [1,2,11,12]

Toxoplasmosis is caused by *T. gondii*, an apicomplexan intracellular protozoan that is found worldwide and affects all warm-blooded species including humans. This parasite has been reported on all continents, whereas the infection rate varies greatly according to environmental factors [13]. Most animals and humans act as intermediate hosts, whereas felids especially domestic cats serve as definitive hosts, shedding oocysts into the environment and consequently facilitating the spread of the *T. gondii* infection [14]. Infection occurs mostly through three routes: transplacental, eating of infected or uncooked meat and drinking of contaminated water [15,16]. Although, milk from diseased animals has been verified as a second source of infection [17].

People, particularly those living in transhumant and pastoral communities, acquire the *T. gondii* infection through consuming raw and undercooked meat from infected animals, as well as raw milk contaminated with oocysts [12]. Toxoplasmosis must be controlled since it can severely reduce the quality of life and potentially cause death in humans and animals. It also has an effect on wildlife and ecological health [18].

In several countries, cases of *T. gondii* infection in *Camelus dromedarius* have been reported [19]. According to Shaapan and Khalil [20], the prevalence of *T. gondii* infection in camels varies significantly by region of the world, it was 3.12% in Iran [21], 15% in Algeria [22], 8.33% in Ethiopia [23], 50.2% in Pakistan [24] and 90.90% in Turkey [25].

Serological tests have been considered as a reliable method to identify *T. gondii* infection in both people and animals. The enzyme-linked immunosorbent assay (ELISA) is an inexpensive and quick test that can analyse a lot of samples in a short time [4]. Certain recombinant proteins of *T. gondii* can be produced in *Escherichia coli* through binding with specific *T. gondii* antibodies and used to identify *T. gondii* antibodies during serodiagnostic tests [26]. In addition, Microneme protein 3 (MIC3) is a key adhesive protein capable of binding to both host and parasite cells [27]. Therefore, it can investigate *T. gondii* efficiently in animals and humans [12,28].

Toxoplasmosis in camels may be a significant disease due to its zoonotic significance and effect on reproductive performance [29]. However, *T. gondii* surveys in domestic animals are uncommon in Egypt compared to many human studies. Only a few studies on *T. gondii* and its risk factors specifically targeted camels, as opposed to the vast majority of *T. gondii* studies in Egypt that concentrated on domestic animals, such as dogs, cats, sheep, goats, and cattle [12,30–32].

Therefore, the aim of this study was to identify the seroprevalence of *T. gondii* in *Camelus dromedarius* in three Egyptian governorates as well as the risk factors for infection.

2. Materials and Methods

2.1. Ethical Statement

The animal ethics committee at Benha University approved all methods used to handle and collect blood samples (Approval No: BUFVTM 06-10-2022). The camel owners gave their permission and informed consent for the collection of samples. All procedures were carried out in conformity with the pertinent regulations and standards of Benha University Faculty of Veterinary Medicine's ethical committee. The ARRIVE guidelines were followed in the execution of this investigation.

2.2. Study Area

The study was carried out in Egypt's governorates of Kafr ElSheikh, Qalyubia, and Marsa Matrouh, Kafr ElSheikh and Qalyubia governorates situated in Nile Delta of Egypt and located geographically at 31°06′42″ N 30°56′45″ E and 30.41° N 31.21° E, (Figure 1). Marsa Matrouh is located in the north-western portion of Egypt, near the Libyan border, at 29.66° N 27.51° E, (Figure 1).

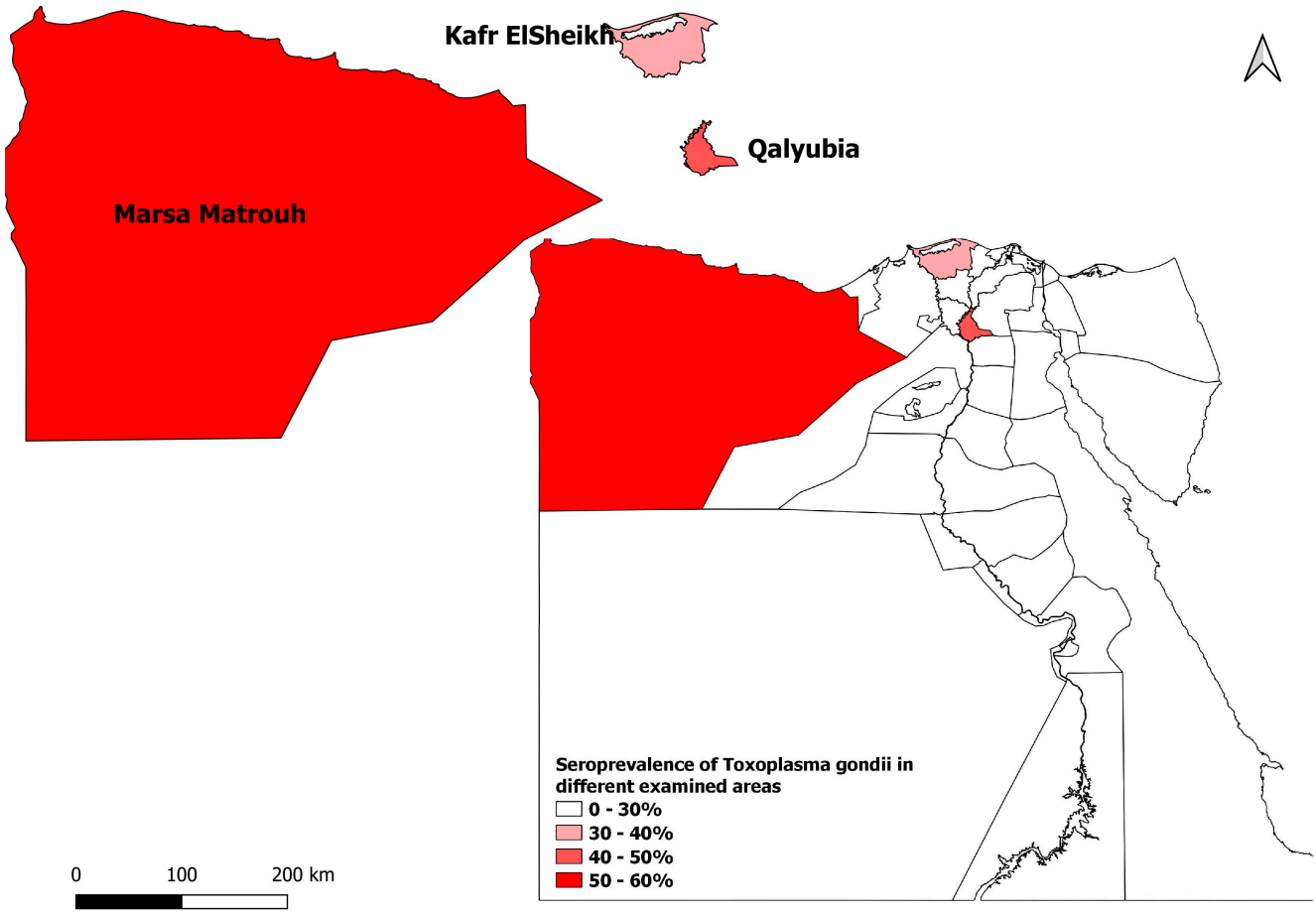

Figure 1. Map showed the reported seroprevalence rates in examined governorates.

The governorates of Kafr ElSheikh and Qalyubia have "desert" climates as classified as BWh by Köppen and Geiger. The average yearly temperature is 22 °C, with rainfall ranging from 100 to 200 mm in the winter. According to Köppen climatic classification, Marsa Matruh has a hot desert climate with dry summers (BWhs). However, winters are warm and generally wet, and summers are moderately hot and humid due to winds from the Mediterranean Sea. Summers are sunny and dry, while winters have moderate rain and cloud cover. Marsa Matruh has the coolest summer days of any Egyptian city or resort, though not much cooler than other northern coastal locations.

The study areas were selected due to the number of dromedaries in the area as well as the large rise in local community consumption of camel meat and milk [33].

2.3. Sampling and Data Collection

A cross-sectional study was conducted in three study locations from January to December 2020 to investigate antibodies against *T. gondii* in examined camels. The following formula suggested by Thrusfield [34] was used to calculate the sample size.

$$n = \frac{1.96^2 \times pexp(1 - pexp)}{d^2}$$

where n is number of required samples, $pexp$ is the a predicted prevalence which was 64.51% according to Khattab, et al. [30] and d is desired absolute precision for a 95% confidence level.

A total of 390 blood samples (10 mL) were randomly collected from individual camels' jugular veins using disposable needles and plain tubes and transferred in an ice box to the veterinary diagnostic laboratory, Faculty of Veterinary Medicine, Benha University. Blood

samples were centrifuged at 1400× *g* for 10 min to separate sera, which were then stored at −20 °C until serological analysis.

The camels studied were of both sexes (male and female) and were divided into three age groups (≤4, 4–8, and >8 years). Camels under the age of four are regarded as young, adults are between the ages of four and eight, while camels older than eight are regarded as elder. All animals had additional data recorded on contact with other small ruminants, abortion history, and parity. Camel grazing and reproduction are permitted without restriction. Most of camels feed mostly on grasses and barseem with few concentrates, while the drinking depends mainly on communal water source. The surveyed camels suffered clinically from anorexia, weight losses and reduction in fertility rate.

2.4. Serological Examination

Anti-*T. gondii* IgG antibodies were investigated using indirect ELISA multispecies diagnostic kit (ID VET Innovative Diagnostic, ID Screen, Montpellier, France), with a sensitivity and specificity of 100% and 96%, respectively [22]. The test was performed following the manufacturer's instructions. The plate's optical density was determined at 450 nm using an ELISA reader (AMR-100, AllSheng, China). The P30 *T. gondii* antigens were used to coat the 96-well plate; later, peroxidase conjugate was added to aid the antigen–antibody complex to develop. Each test comprised both positive and negative controls, which were provided by the manufacturer, and an animal was considered positive when the serum had an OD% of more than or equal to 50%.

2.5. Statistical Analysis

The statistical software SPSS version 24.0 was used for all statistical analyses (SPSS Inc., Chicago, IL, USA). The chi square test (χ^2) was used to evaluate the seroprevalence and its association risk factors as an independent categorical variable with *T. gondii* seropositivity. The strength of the association between risk factors and *T. gondii* seropositivity was determined using logistic regression analysis. In a multivariable logistic regression model, noncollinear variables with $p < 0.20$ in univariable analysis at the 95% confidence level were entered. Correlation matrix between significant variables from the univariable analyses revealed a high correlation between sex, age and parity. The statistical significance level was at $p < 0.05$.

3. Results

Out of 390 sera of camels examined using ELISA, 183 camels (46.9%; 95%CI: 42.02–51.88) had antibodies against *T. gondii*. Moreover, locality, sex, age, contact with sheep and goats, history of abortion, and parity were all found to have a significant impact on *T. gondii* seropositivity ($p < 0.05$) in camels from Egypt (Table 1). There was a statistically significant variation in seroprevalence between the geographical areas, with Marsa Matrouh having the highest seroprevalence (54.7%; 95% CI: 46.69–62.42, p value = 0.035) and Kafr ElSheikh having the lowest (39.2%; 95% CI: 30.9–48.11), Figure 1. The seroprevalence was higher in older camels >8 years (63.8%, 95% CI: 55.3–71.6, p value < 0.0001) than other age groups and in females (54.6%; 95% CI: 49.08–60.01, p value < 0.00001) than males. Furthermore, camels exposed to small ruminants had higher seropositivity (64.2%, 95% CI: 55.27–72.19, p value < 0.00001), particularly in cases of abortion history (68.3%, 95% CI: 55.76–78.69, p value < 0.00001) and parity greater than four (72.6%, 95% CI: 64.52–79.41, p value < 0.00001), Table 1.

In the univariable analysis, the variables with a $p < 0.25$ were subjected to the multivariable logistic regression model. The results revealed that *T. gondii* infection was three times more likely in camels living in Marsa matrouh (OR = 2.02, 95% CI: 1.02–4.01) than in Qalyubia (OR = 1.13, 95% CI: 0.56–2.24); seven times more likely in females (OR = 7.07, 95% CI: 3.25–15.39); and five times more likely in adult camels > 8 years old (OR = 5.28, 95% CI: 2.35–11.90), Table 2. Additionally, the probability of *T. gondii* infection was four

times more in females with a history of abortion (OR = 3.84, 95% CI: 1.76–8.36) and a parity of more than four (OR = 17.72, 95% CI: 2.35–133.57), Table 2.

Table 1. Univariable analysis of risk factors associated to *T. gondii* infection in camels.

Variable	Total of Tested Camels	No of Positive	No of Negative	% of Positive	95% CI	Statistic
Locality						
Kafr ElSheikh	120	47	73	39.2	30.9–48.11	χ^2 = 6.688 df = 2 p = 0.035 *
Qalyubia	120	54	66	45.0	36.39–53.92	
Marsa Matrouh	150	82	68	54.7	46.69–62.42	
Sex						
Male	75	11	64	14.7	8.39–24.39	χ^2 = 38.793 df = 1 p < 0.0001 *
Female	315	172	143	54.6	49.08–60.01	
Age						
<4	70	15	55	21.4	13.44–32.39	χ^2 = 33.582 df = 2 p < 0.0001 *
>4–8	190	85	105	44.7	37.84–51-84	
>8	130	83	47	63.8	55.3–71.6	
Contact with sheep and goats						
Yes	120	77	43	64.2	55.27–72.19	χ^2 = 20.694 df = 1 p < 0.0001 *
No	270	106	164	39.3	33.62–45.2	
History of abortion						
Yes	60	41	19	68.3	55.76–78.69	χ^2 = 13.051 df = 1 p < 0.0001 *
No	330	142	188	43.0	37.8–48.42	
Parity						
1	10	2	8	20.0	5.67–50.98	χ^2 = 32.747 df = 2 p < 0.0001 *
>1–4	170	72	98	42.4	35.17–49.87	
>4	135	98	37	72.6	64.52–79.41	
Total	390	183	207	46.9	42.02–51.88	

* p < 0.05 indicates the result is significant. CI: confidence interval, χ^2: chi-square.

Table 2. Multivariable logistic regression for associated risk factors to *T. gondii* infection in camels.

Factor	B	S.E.	OR	95% C.I. for OR		*p* Value
				Lower	Upper	
Locality						
Qalyubia	0.118	0.352	1.13	0.56	2.24	0.737
Marsa Matrouh	0.701	0.350	2.02	1.02	4.01	0.045
SexFemale	1.957	0.397	7.07	3.25	15.39	<0.0001
Age						
>4–8	0.556	0.407	1.74	0.79	3.87	0.172
>8	1.665	0.414	5.28	2.35	11.90	<0.0001
Contact with sheep and goats						
Yes	1.348	0.306	3.85	2.11	7.01	0.001
History of abortion						
Yes	1.345	0.397	3.84	1.76	8.36	<0.0001
Parity						
>1–4	1.519	1.020	4.57	0.62	33.74	0.137
>4	2.875	1.031	17.72	2.35	133.57	0.005

B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval.

4. Discussion

Toxoplasmosis affects a wide range of hosts, including animals and humans. Using sensitive molecular methods, it was discovered in milk and raw meat of small ruminants in Upper Egypt [12]. The prevalence of *T. gondii* infection in camels has increased in recent years due to the importance of domestic ruminants in parasite transmission, whether through direct contact or intake of animal meat products [22].

According to previous studies, the ELISA test was used in the current study since it is a more sensitive and specific approach for sero-epidemiological examinations of *T. gondii* infection in animals [30]. Despite the economic importance of camels in Egypt, there is a general dearth of awareness regarding camel illnesses, particularly toxoplasmosis. To the best of our knowledge, this is the first study to examine the risk factors for *T. gondii* infection in Egyptian camels.

The overall seroprevalence of this study was 46.9%, which was lower than earlier reported rates for *T. gondii* in Egyptian camels using ELISA by Toaleb, et al. [35] 66.7% and Khattab, et al. [30] 64.51%. In contrast, the reporting seroprevalence was higher than that found in the United Arab Emirates (22.4%) by Abu-Zeid [36]; in Turkey (40%) by Gebremedhin, et al. [16]; in Sudan (20%) by Khalil and Elrayah [37]; in Saudi Arabia (6.5%) by Al-Anazi [38]; in Iran (3.12%) by Dehkordi, et al. [21]; in Algeria (45%) by Nasreen, et al. [39]; and in Ethiopia (8.33%) by Gebremedhin, et al. [9].

Although it may be difficult to compare the prevalence across countries, the differences in reported seroprevalences could be attributed to a variety of factors including the density of domestic cats or wild felids, livestock management practices, soil type and climate conditions, sample size, serum dilutions, serological test limits and sensitivity differences, and stress factors [16,37,38,40–44].

However, lack of routine culling programmes, poor veterinary care, and the migration of camels to sub-arid areas in quest of better ranges could all be contributing causes to the incidence of *T. gondii* infection in dromedaries in this area of Egypt. Additionally, it might be associated with local ownership transfers, agricultural growth, and the accumulative impacts of camel ageing [29].

Moreover, camels from Marsa Matrouh were approximately three times (OR = 2.82) more likely than camels from Kafr Elsheikh governorate to be seropositive to *T. gondii* infection in this study. This variance could be attributable to changes in environmental temperatures and moistures [45–48], as well as a higher probability of camels in the Marsa Matrouh governorate coming into contact with small ruminants, which may have led to the significantly higher seroprevalence [49,50].

The seroprevalence of *T. gondii* infection was relatively lower in the current study (46.9%) compared to a prior study conducted by Khattab, et al. [30] in the north-west of Egypt (64.51%), which could be the result of a number of factors, including the small animal population, low cat density, low human population in study areas and rearing of camels in border areas of Egypt [51].

Female camels had higher seroprevalence rate for *T. gondii* than male camels ($p < 0.0001$), which could be owing to a reduced physiological or immunological status because of pregnancy and lactation stress. This finding is in accordance with Gebremedhin, et al. [9] and Hussein, et al. [52]; they found a strong association between seropositivity to *T. gondii* and the sex of camels. Contrary to these findings, Silva, et al. [53] observed higher prevalence for toxoplasmosis in males than females while other authors reported no variation between the two genders [23]. This contradicts findings found in previous studies of camels from Egypt [54], Saudi Arabia [52], Sudan [55], and China [56].

The analysis of present data provide evidence for older camels (>8 years) having greater seroprevalence for *T. gondii* (OR = 5.28; $p < 0.0001$) than younger camels ($\leq$4 years), where the seroprevalence increased in correlation with age of camels. In comparison to younger animals, older animals had more probability of being exposed to various risk factors for *T. gondii* infection.

This finding is consistent with studies from Ethiopia and Algeria, which found that adult camels have a greater seroprevalence than young camels [9,39]. Since it was noted that animals that live longer may be more likely to be exposed to various sources of *T. gondii* parasites, this could be explained by long exposure of an adult animal to parasite infection and older animals are more likely to be a source of *T. gondii* [23,57].

Moreover, the seropositivity to *T. gondii* increased significantly in camels living in contact with small ruminants, as similarly concluded by Abdallah, et al. [29]. This could be explained by the fact that small ruminants are susceptible to *T. gondii* and harbour the parasite's bradyzoite for life [58]. Additionally, there may be a connection between the increased seropositivity of *T. gondii* infection in camels and the outside storage of animal feeds that are accessible to cats and small ruminants [49].

According to the findings, *T. gondii* seroprevalence is significantly higher in camels with a history of abortion and a high number of parities, which is in agreement with previous reports of Gebremedhin, et al. [9]. This could be due to increasing exposure to various *T. gondii* infection sources when the parity number rises [59,60].

5. Conclusions

The present study's findings confirm a high frequency of *T. gondii* infection in camels living in the three studied locations. Locality, sex, age, history of abortion and number of parity were identified as potential risk factors for *T. gondii* infection. Thus, the greater seroprevalence observed in camels used as a food source highlighted the possible danger of *T. gondii* infection conveyed to humans through meat intake. Consequently, people's awareness of *T. gondii* infection transmission and prevention should be raised through education, and further research on the disease's influence on food animal production should be conducted.

Author Contributions: Conceptualization, A.S., M.A.M., A.A. and M.H.W.; methodology, A.S. and A.A.; formal analysis, A.S.; investigation, A.S., M.A.M., A.A. and M.H.W.; resources, A.S., A.A. and M.H.W.; data curation, A.S. and M.H.W.; writing—original draft preparation, A.S., M.A.M., A.A. and M.H.W.; writing—review and editing, A.S., M.A.M., A.A. and M.H.W.; visualization, A.S., M.A.M., A.A. and M.H.W.; supervision, A.S.; project administration and funding acquisition, A.S., M.A.M., A.A. and M.H.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal ethics committee at Benha University approved all methods used to handle and collect blood samples (Approval No: BUFVTM 06-10-2022). The camel owners gave their permission and informed consent for the collection of samples. All procedures were carried out in conformity with the pertinent regulations and standards of Benha University Faculty of Veterinary Medicine's ethical committee.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data that were generated or analysed during this study are included in this published article.

Acknowledgments: The authors are grateful to the Deanship of Scientific Research, king Saud University for funding through the Vice Deanship of Scientific Research Chairs.

Conflicts of Interest: The authors declare no conflict of interest.

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