**Iraqi Journal of Veterinary Sciences**

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# *Toxoplasma gondii* infection in aborted women and sheep in the governorates of El-Beheira and Alexandria, Egypt: A sero-immunological and molecular study

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**Article information**

***Article history:***
Received 03 January, 2025
Accepted 30 April, 2025
Published 19 May, 2025

***Keywords***:
*Toxoplasma gondii*
Sero-immunology
PCR
Aborted women
Sheep

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**Abstract**

Abortion results from a *Toxoplasma gondii* infection in both humans and mammalian animals, causing substantial losses in terms of quality-adjusted life years and financial consequences. The incidence of toxoplasmosis in the current study of aborted women and sheep was determined using serological, immunological, and molecular assays. Blood samples were taken from sheep that appeared to be healthy and from women who had aborted in the governorates of Alexandria and El-Beheira on the west coast of Egypt. The specific immunogenetic bands in the crude tachyzoite antigens from both RH and local *T. gondii* strains were characterized using SDS-PAGE and immunoblotting assays. An indirect ELISA was performed to detect the prevalence in aborted women and sheep. The confirmatory molecular identification of toxoplasmosis was adopted using conventional polymerase chain reaction (cPCR). The results revealed that the two RH and local *T. gondii* antigens showed extensive electrophoretic similarity and identified two common immunogenic bands (67 and 58 kDa) with naturally infected human and sheep sera with *T. gondii*. The rate of toxoplasmosis seroprevalence recorded by ELISA in aborted women was 60.8% (76/125), while in sheep, it was 67.3% (66/98). Verified PCR results of toxoplasmosis in aborted women and sheep revealed the expected positive bands, 194 bp and 161 bp, using B1 and Tox-9 & Tox-11 gene primers, respectively. This study concluded that the higher *T. gondii* prevalence might be responsible for abortion and economic losses in humans and sheep in our studied region. These findings reinforce the necessity of more epidemiological studies on toxoplasmosis and public health education initiatives.

DOI: 10.33899/ijvs.2025.156331.4072, 

## Introduction

The parasite zoonosis that is most common worldwide is *Toxoplasma gondii* (*T. gondii*) (1). Both humans and animals can contract toxoplasmosis from this obligatory intracellular worldwide apicomplexan protozoan parasite (2). All warm-blooded animals, including humans and birds, exist as intermediate hosts for the facultative heteroxenous lifecycle of the *Toxoplasma* parasite, whereas the definitive hosts are domesticated cats and other feline family members (3). Although *T. gondii* infections affect over one-third of all people on the planet, toxoplasmosis symptoms are not very prevalent (4). The disease can infect humans if they consume food or water polluted with cat feces that contain sporulated oocysts or by eating raw or improperly heated meat that has tissue cysts (bradyzoites) (5). Humans can also contract *T.*

*gondii* by consuming unpasteurized milk that contains tachyzoites (6). In the majority of immunocompetent, apparently healthy adult individuals, *T. gondii* infections are not noticeable; however, they could result in clinical symptoms that last for months, including fever, lymphadenitis, influenza-like illness, and muscle aches (7). Conversely, suppose the mother and female animals contract T. gondii while they are pregnant. In that case, the fetuses may have a severe form of toxoplasmosis (8). In such circumstances, the presence of *T. gondii* infection causes severe clinical manifestations, including retinochoroiditis, microcephaly, stillbirth, hydrocephalus, cerebral calcification, psychosis, and many schizophrenic episodes (9). Furthermore, *T. gondii* infection may linked to neuropsychiatric diseases, encephalitis, meningitis, and neurobehavioral abnormalities in immune-compromised individuals, including cancer patients, organ transplant recipients, HCV and HIV infected individuals (10). One of the most common infectious diseases that inhibit reproduction is sheep toxoplasmosis since placentitis, stillbirth, and miscarriage may each result in large economic losses (11). The main way that sheep contract the disease is by consuming *T. gondii* oocysts while grazing in contaminated environments (12). Since sheep are more inclined than other farm animals to become infected, multiple studies have demonstrated elevated *T. gondii* seroprevalence in sheep from different parts of the world (13-15). Where people frequently eat sheep's mutton, such as in Egypt, a public health alert should be issued; it has been noted that the incidence of *T. gondii* infection in Egyptian sheep fluctuates (16-18). The majority of cases of *toxoplasma* infection are asymptomatic, even at this point; there are several laboratory methods for diagnosing the infection. The most popular methods for identifying toxoplasmosis in sheep and humans are serological tests that recognize the anti-*T. gondii*-specific immunoglobulin, IgM, and IgG in serum samples (19). The IgG avidity assay has been used to identify the anti-*T. gondii*-specific antibodies in both pregnant and aborted women (20), the Indirect Hem Agglutination Test (IHAT) (21), and the Latex Agglutination Test (LAT) (16). Furthermore, antibodies against *T. gondii* were detected in sheep using a modified agglutination test (22) and Enzyme-Linked Immunosorbent Assay (23). Polymerase Chain Reaction (PCR) in blood samples from aborted women (24) and sheep (25) can molecularly identify *T. gondii*.

Consequently, it is imperative to collect essential data to have a deep understanding of the effects of toxoplasmosis in Egypt. Thus, the objectives of the current investigation were to apply an ELISA assay followed by the Immunoblotting Technique to ascertain the sero-immunological detection of *T. gondii* among sheep and women who were aborted in Egypt's El-Beheira and Alexandria Governorates, as well as to molecularly establish.

## Materials and methods

### Ethical approval

Under registration number 19-145, the experiment was conducted in compliance with the institutional norms of the Animal Research Committee of the National Research Center. NRC, Egypt, project number 1201034.

### Study area and samples

Between February 2024 and September 2024, an epidemiological study was conducted to determine whether toxoplasmosis occurred in apparently healthy sheep and women who reported spontaneous miscarriages. 98 blood samples were drawn from apparently healthy sheep belonging to different herds in the El-Beheira Governorate. In terms of humans, 125 blood samples were taken from women who had abortions and were admitted to the labor and delivery units of El Shatby Hospital for Obstetrics and Gynecology in Alexandria, Egypt. Serum samples were separated, placed in 1.5 ml labeled Eppendorf tubes, and then stored at -20° C until appropriate serological examination and assessment were completed.

### Antigens preparation

The infectious stages of *T. gondii* were locally isolated from the pooled flesh sample (heart and diaphragm) obtained from slaughtered sheep in accordance with the Shaapan and Ghazy method (26). Both locally isolated and virulent (RH) *T. gondii* strains were kept in the Department of Zoonotic Diseases, National Research Centre, by serial passage in mice according to the procedure of Hassan *et al.* (27). According to Hassan *et al.* (28), the antigens of *T. gondii* tachyzoites from the two isolates were prepared by repeatedly freezing and thawing the tachyzoites to break down the parasite wall, sonicating them, centrifuging them for 40 minutes at 4°C at 16,000 rpm, and collecting the supernatants. The protein content of the *T. gondii* tachyzoites antigens was determined using the Lowry *et al.* (29) technique.

### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The RH and regionally isolated strains of *T. gondii* crude antigen proteins were mixed separately with a reducing buffer containing 5% 2-mercaptoethanol, and they were subsequently electrophoresed on 10% SDS-PAGE using the Laemmli method (30). The relative molecular weights of the bands were ascertained by electrophoresing them on the same gel with pre-stained markers (GeneDirex BLUltra, USA).

### Western blot (Immunoblotting)

In order to identify the specific immunogenic bands identified in the crude antigens of RH and isolated *T. gondii* tachyzoite strains, immunological detection of protein bands

on nitrocellulose membranes was carried out using naturally infected human and negative sheep and human sera, as described by Hassanain *et al.* (31). To identify immuno-reactive bands, 4-chloro-1-naphthol (Sigma-Aldrich) was utilized as a substrate. Utilizing Bio-Rad's Molecular Imager Gel DocTM XR lab software, the two-nitrocellulose membranes were assessed.

**Enzyme-Linked Immune-sorbent Assay (ELISA)**

Using tachyzoite antigens of the local strain of *T. gondii*, indirect ELISA was performed to detect the presence of *T. gondii* IgG in human and sheep serum. The assay was carried out using the procedures outlined by El-Fadaly *et al.* (32). The optimal concentrations of the antigens, serum samples, and conjugates were determined using checkerboard titration (33). Optical density (OD) values are used to express the results. Three times the standard deviation plus the mean OD values of the negative control sera were used to estimate the cut-off (34). Sero-positive results were defined as OD greater than the cut-off value.

**Molecular identification**

In accordance with the instructions provided by the DNA gel extraction kit (NORGRN BIOTEK, Cat. No. 13100), the conventional polymerase chain reaction (cPCR), which was employed for the genetic characterization of the Toxoplasma, was utilized to extract genomic DNA from the obtained whole blood samples. At the same time, the tachyzoites of the *Toxoplasma gondii* RH strain were employed to harvest DNA for use as positive control. The eluted DNA's concentration and purity were assessed using spectrophotometry, and it was stored at -80ºC for subsequent processing. For PCR, two distinct sets of primers based on the B1 genes and the 529 bp repeating region were employed independently (Table 1).

Table 1: The different primer sets used for molecular detection of toxoplasmosis

| Genes | Primer Name | Primer sequence (5'-3') | Annealing (Cº) | Expected size |
|---|---|---|---|---|
| B1 genes | Tox B1 Forward<br>Tox B1 Reverse | TCG GAG AGA GAA GTT CGTCGC AT<br>AGC CTC TCT CTT CAA GCA GCG TA | 55 | 192 bp |
| 529 bp repeat region | Tox-9 forward<br>Tox-11 reverse | TGG AGG AGA GAT ATC AGG ACT<br>GCG TCG TCT CGT CTA GAT CG | 60 | 162 bp |

**Results**

**Electrophoretic profile of the two antigens**

Virulent (RH) and locally isolated *T. gondii* antigens exhibited a complex separation pattern, and the two antigens' electrophoretic similarities were particularly strong in terms of 96, 74, 67, 58, and 41 kDa. However, only virulent (RH) antigens exhibited exclusive molecules, such as 30 kDa, 27 kDa, and 14 kDA (Figure 1, lane A), and 35 kDa, 25 kDa, 21 kDa, and 12 kDa, which were exclusively linked to locally isolated *T. gondii* antigens (Figure 1, lane B).

**Sero-Diagnosis of Human and Sheep Toxoplasmosis**

The isolated local *T. gondii* antigen demonstrated a higher infection rate of 67.3% (66/98) in sheep (Table 2 and Figure 2), which was higher than the 60.8% (76/125) seen in aborted women serum samples (Figure 3).

Table 2: Sero-diagnosis of human and sheep toxoplasmosis by Indirect ELISA using locally isolated *T. gondii* sheep strain antigen

| Sera | Examine (n) | Positive (n) | Positive (%) |
|---|---|---|---|
| Human | 125 | 76 | 60.8% |
| Sheep | 98 | 66 | 67.3% |

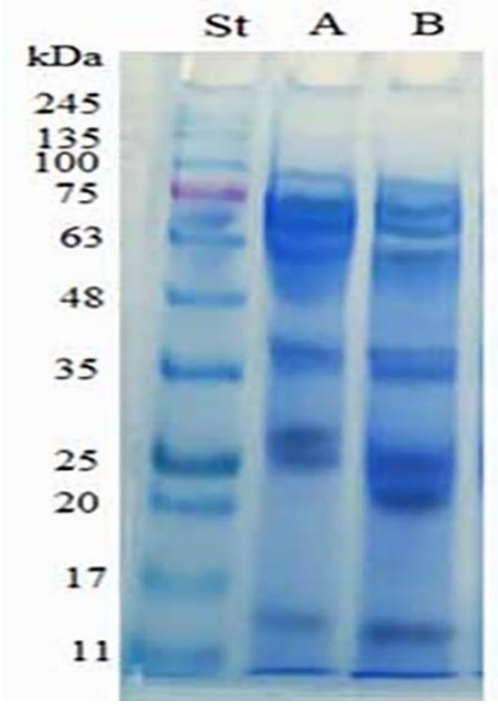


Figure 1: Electrophoretic profile showed comparative SDS-PAGE of virulent (RH) antigen (Lane A) and local isolates of the *T. gondii* antigen (Lane B) and molecular weight standards (Lane St).

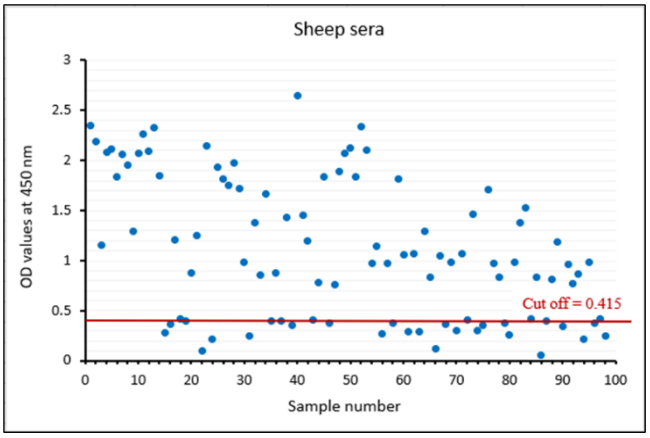


Figure 2: Sheep sera's IgG antibody levels against the locally isolated *T. gondii* antigen.

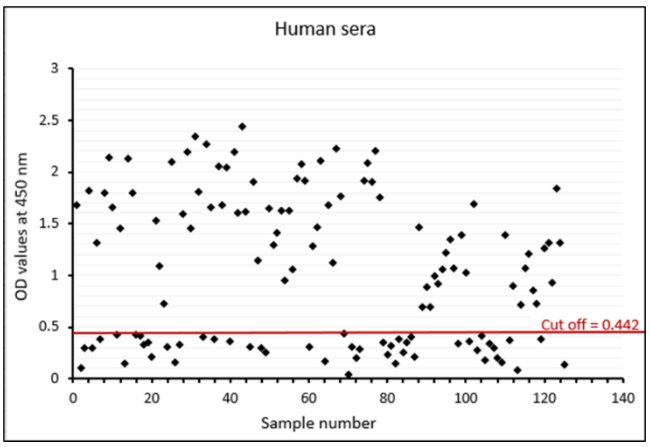


Figure 3: human sera's IgG antibody levels against the locally isolated *T. gondii* antigen.

**Immunogenic bands of virulent (RH) and locally isolated *T. gondii* antigens**

Two bands with molecular weights of 67 kDa and 58 kDa were found using naturally infected sheep sera, while three immunogenic bands with molecular weights of 67, 58, and 27 KDa were found in virulent (RH) antigen by naturally infected human sera (Figure 4). In the locally isolated *T. gondii* antigen using naturally infected human and sheep sera, three immunogenic bands of 67, 58, and 25 KDa (Figure 5). No immunogenic reactive bands were detected in two antigens when using negative sera of humans and sheep (Figures 4 and 5).

**Molecular identification**

Conventional polymerase chain reaction (cPCR) analysis proved the detection of *Toxoplasma gondii* using B1 gene primers at the expected size of 194 bp (Figure 6). Whereas using Tox-9 and Tox-11 primers, *Toxoplasma gondii* was identified at the expected size of 161 bp (Figure 7).

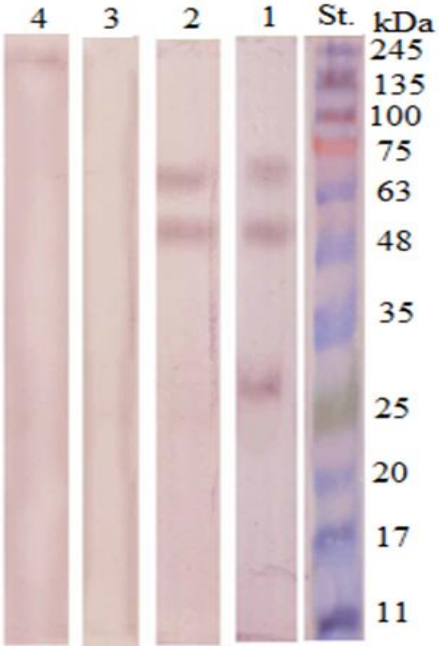


Figure 4: Immune-reactive bands identified in virulent (RH) antigen by immunoblot assay using naturally infected human sera (Lane 1), naturally infected sheep sera (Lane 2), negative human sera (Lane 3), negative sheep sera (Lane 4) and molecular weight standards. (Lane St.).

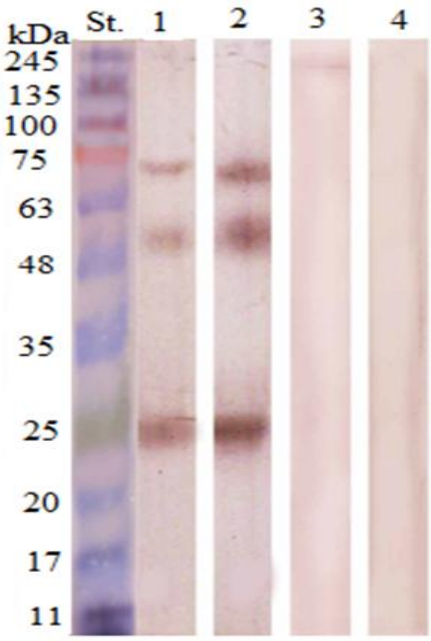


Figure 5: Immune-reactive bands identified in local isolates of *T. gondii* antigen by immunoblot assay using naturally infected human sera (Lane 1), naturally infected sheep sera (Lane 2), negative human sera (Lane 3), negative sheep sera (Lane 4) and molecular weight standards (Lane St.).

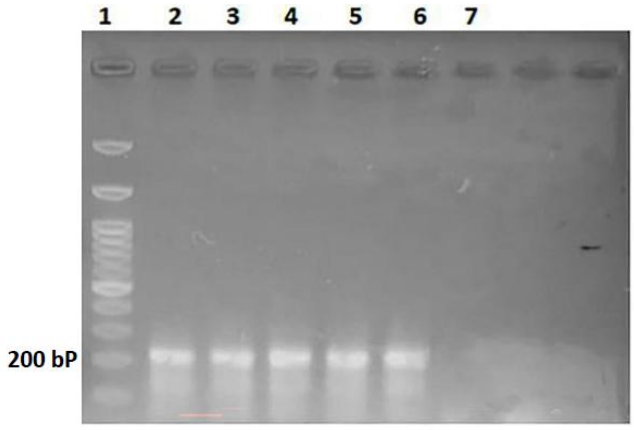


Figure 6: Detection of *Toxoplasma* genome using B1 gene primers. Lane 1, 100 bp DNA ladder; lanes 2 & 3, positive human and lanes 4 & 5, positive sheep (194 bp); lane 6, positive control and lanes 7, negative specimen.

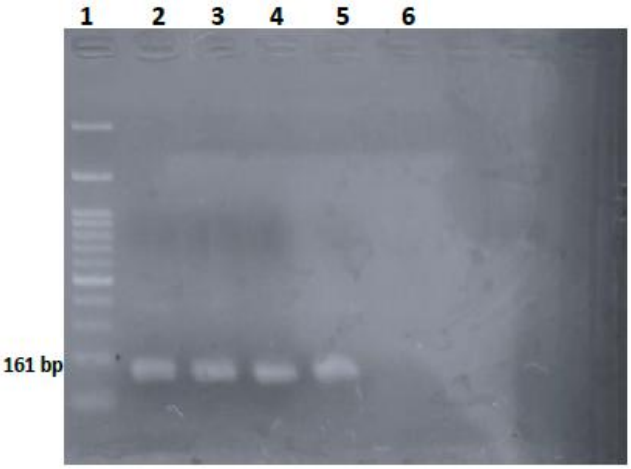


Figure 7: Detection of *Toxoplasma* genome using Tox-9 and Tox-11 primers. Lane 1, 100 bp DNA ladder; lanes 2 and 3; positive human and lanes 4, positive sheep (161 bp); lane 5, positive control and lanes 6, negative specimen.

**Discussion**

In the current study, the electrophoretic profile of the two RH and local *T. gondii* antigens exhibited extensive similarity, and when exposed to western blot analysis probed with *T. gondii* positive sera from human and sheep, they identified two common immunogenic bands of 67 and 58 kDa. Numerous investigations have been conducted in recent years to identify the proteins of *T. gondii* tachyzoites, particularly immunoreactive proteins (35). These research efforts have concentrated on choosing immunoreactive and protective antigens that might be employed in accurate diagnosis and vaccine preparation of toxoplasmosis (17). *T. gondii* matrix antigens of 67 and 58kDa protein, which is widely expressed in both RH and local *T. gondii* tachyzoites antigens, were assessed as potential candidates for the development of a diagnostic reagent for ovine toxoplasmosis (36). Human toxoplasmosis was successfully diagnosed using a 65 kDa fraction that was extracted from horse-derived *T. gondii* tachyzoites (28). Additionally, a 65-kDa fraction of the local camel *T. gondii* strain tachyzoites that were included in the affinity-isolated fraction was effectively used to diagnose toxoplasmosis in cattle (37). The antisera used in each study may be the reason for the discrepancy between the immunogenic bands of the currently evaluated antigens used and those found in previous studies (38-40).

The quality of the antigen used, the type of diagnostic potentials, and the accessibility and affordability of antigen preparation are the primary determinants of the serological diagnosis's accuracy and economy (23,41). The current study uses an easily prepared and relatively low-coasted locally isolated *T. gondii* antigen to detect the seroprevalence rate of toxoplasmosis, which was 60.8% (76/125) in women who had abortions and 67.3% (66/98) in sheep from El-Beheira and Alexandria Governorates, Egypt. Toxoplasmosis is not the only prevalent cause of women's habitual abortions, even if women infected with *T. gondii* can have abortions (8). Toxoplasmosis has been detected in many Egyptian sheep and women who had aborted fetuses; the seroprevalence is almost higher in sheep 61.76% and pregnant women 63.79% from the Menoufia Governorate in the Delta (42). In contrast, in the El-Minya Province, pregnant women (22.9%) and sheep (39.1%) had lower seroprevalence (16). Previous studies found that 46% of sheep and 60.7% of aborted women in the Cairo governorate tested positive for Toxo IgG by ELISA (22,43). Rather than a lower frequency in the urbanized Cairo governorate compared to the rural governorates, the previously observed disparities in seroprevalence may be due to variations in ecological factors, the serological assays utilized, sample size, sheep breed, place, and time of sampling (44).

There have been several published studies on toxoplasmosis in pregnant Egyptian women; however, they frequently have tiny sample sizes and do not include information on the populations the researchers were trying to study. Because of the sample size, diagnostic technique employed, and living conditions of the women evaluated, there are few statistics on seroconversion before and during pregnancy, and results cannot be compared across publications (45). The Lower (North) Egyptian governorates and Cairo had higher rates of *T. gondii* infection than the Upper (South) Governorates; these disparities suggest that socioeconomic and geographic factors may influence *T. gondii* exposure in Egypt (46). Multivariate regression study shows that eating raw or undercooked food, especially sheep meat, interacting with cats, and working in agriculture are all-important risk factors for *T. gondii* seropositivity (5,47).

PCR analysis in this study proved that the detection of *Toxoplasma gondii* using B1 gene primers and Tox-9 and Tox-11 gene primers at the expected size of 194 bp and 161 bp, respectively. The PCR technique has shown promise in a number of applications, such as allelic discrimination, gene expression, and pathogen detection (48). Numerous PCR-based methods have been created to diagnose toxoplasmosis utilizing a range of clinical specimens, such as tissue biopsies, blood, amniotic fluid, and cerebrospinal fluid (49,50). During the active phase of *T. gondii* infection, the pregnant mother may test negative, hence preventing detection of the high risk of neonatal toxoplasmosis (20). DNA from *T. gondii* was found in placentas or other unidentified products of conception in a few of the reports. Once more, strict controls are necessary for PCR accuracy in order to reduce contamination. It is important to highlight that *T. gondii* DNA in the placenta does not necessarily indicate a prenatal infection (51-53).

## Conclusion

The present study suggests that women who experienced several miscarriages and lambs infected with *T. gondii* had higher levels of IgG. This study highlights the importance of *T. gondii* blood tests, health education programs, and preventative measures such as appropriate meat handling, eating, and cleaning practices in lowering the prevalence of these disorders. Additionally, it recommended that additional comprehensive research be done to validate the results and offer recommendations for public health programs.

## Acknowledgments

The authors would like to express their appreciation to their colleagues in the National Research Center's Department of Zoonotic Diseases for helping them finish this work.

## Conflict of interest

The authors have disclosed no conflicts of interest.

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# عدوى داء المقوسات في النساء المجهضات والأغنام في محافظتي البحيرة والإسكندرية، مصر: دراسة مصلية مناعية وجزيئية

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**الخلاصة**

تسبب عدوى المقوسة الكوندية الإجهاض لدى البشر وكذلك الحيوانات الثديية، مما يؤدي إلى خسائر كبيرة من حيث الصحة وسنوات الحياة وحسب العواقب المالية. في الدراسة الحالية، تم تحديد معدل حدوث داء المقوسات (المقوسة الكوندية) في النساء والأغنام المجهضة باستخدام الاختبارات المصلية والمناعية والجزيئية. وقد تم أخذ عينات الدم من الأغنام التي بدت صحية ومن النساء اللاتي أجهضن في محافظتي الإسكندرية والبحيرة في الساحل الغربي لمصر. وتم تحديد الأشرطة المناعية المحددة في مستضدات المقوسة الكوندية الخام من كل من السلالات الضارية (ار اتش) والمحلية باستخدام اختبارات الأشرطة الكهربائية والطبع المناعية وتم إجراء اختبار الاليزا غير المباشر للكشف عن انتشار داء المقوسات لدى النساء والأغنام المجهضة. وقد استخدم التعريف الجزيئي التأكيدي على داء المقوسات باختبار تفاعل البلمرة المتسلسل التقليدي. وأوضحت النتائج أن المستضدين الضاري (ار اتش) والمستضد المحلي لطفيل المقوسة الكوندية أظهرا تشابهًا كهربائيًا واسع النطاق وحددا نطاقين مناعيين شائعين (٦٧ و٥٨ كيلو دالتون) في مصل الإنسان والأغنام المصابة بشكل طبيعي في حين كان معدل انتشار داء المقوسات المصلي المسجل بواسطة اختبار الاليزا في النساء المجهضات ٦٠,٨٪ (١٢٥/٧٦)، بينما كان في الأغنام ٦٧,٣٪ (٩٨/٦٦). وكشفت نتائج تفاعل البلمرة المتسلسل تأكيدا لداء المقوسات في النساء المجهضات والأغنام عند النطاقات الإيجابية المتوقعة، ١٩٤ و١٦١ زوجًا قاعديًا باستخدام بادئات جين ب١ وتكسو ٩ مع تكسو ١١ على التوالي. وخلاصة هذه الدراسة أن ارتفاع معدل انتشار الإصابة ب داء المقوسات قد يكون مسؤولاً عن الإجهاض والخسائر الاقتصادية لدى البشر والأغنام في المنطقة قيد الدراسة. وتدعم هذه النتائج ضرورة إجراء المزيد من الدراسات الوبائية حول داء المقوسات وحملات التثقيف الصحي العام.