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## SEROLOGICAL STUDIES OF *TOXOPLASMA GONDII* INFECTION IN CAMELS (*CAMELUS DROMEDARIUS*) (With One Table and One Figure)

By

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دراسات مصلية لإصابة الجمال (وحيدة السنام) بطفيل التوكسوبلازما جوندى

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طفيل التوكسوبلازما جوندى واحد من أهم الطفيليات المشتركة واسعة الانتشار الجغرافي على مستوى العالم. جميع الحيوانات تعتبر عائل وسيط ماعدا العائلة القطيه فهي تعتبر العائل الأساسي للطفيل. ما بين عامي ٢٠٠٩/٢٠١٠ تم جمع عدد ٤٨٢ عينة سيرم (١٨٢ ذكر - ٣٠٠ أنثى) من الجمال (وحيدة السنام) وكانت أعمارها من ٣-٦ سنوات من خمس محافظات مختلفة من المملكة العربية السعودية، حيث تم إجراء اختبار الاليزا الغير مباشر وذلك للكشف عن الأجسام المضادة لطفيل التوكسوبلازما جوندى في عينات السيرم التي تم جمعها. أظهرت نتائج هذه الدراسة إن عدد ٢١٩ (من جملة ٤٨٢) عينه سيرم كانت ايجابية لوجود الأجسام المناعية المضادة الدالة على الإصابة بطفيل التوكسوبلازما جوندى وذلك بنسبة (٤٥,٤٤%) وكانت نسبة الإصابة في الإناث (٤٧%) اعلي منها في الذكور (٤٢,٨٦%) ولوحظ أن نسبة الإصابة في محافظة الرياض والخرج (٥٠,٦٨% - ٤٧,١٥%) كانت اعلي من المحافظات الثلاث الأخرى وهي وادي الدواسر (٤١,٨٤%) وضرماء (٤٠,٥٨%) والسليل (٣٨,٤٦%) علي الترتيب. هذه الدراسة النوعية توضح مدى إصابة الجمال بطفيل التوكسوبلازما جوندى على نطاق واسع بالمملكة السعودية وذلك مما يعكس أثرها الاقتصادي وأهميتها على الصحة العامة للإنسان لا سيما وإن البدو يستهلكون الحليب والكبد الغير ناضج من هذه الحيوانات وهذا مما يستحق نظرة فاحصة إلى كيفية إصابة الجمال وطريقة اكتسابها العدوى بطفيل التوكسوبلازما جوندى خصوصا في بيئتها القاحلة.

### SUMMARY

*Toxoplasma gondii* is one of the important zoonotic parasites of worldwide zoological and geographical distribution. All animal species act as intermediate hosts to the *Toxoplasma gondii*, except feline species which act as a definitive host. A total of 482 serum samples (182 male and 300

female) were collected during 2009/2010 from native camels (3-6 years), represented five different provinces of Saudi Arabia to monitoring serologically for *Toxoplasma gondii* antibodies by the indirect enzyme linked immuno-sorbant assay (ELISA). A total of 219 (45.44%) camel sera were serologically positive for toxoplasmosis. The prevalence was higher in female (47%) than male (42.86%). The prevalence was high in Al-Riyadh and Alharig (50.68% and 47.15%) comparing to other areas: Wady Al-Dawaser (41.84%), Dar-maa (40.58%) and Al-Solyel (38.64%) respectively. This study suggests widespread infection with *Toxoplasma gondii* among camels, a finding that warrants a closer look into the possible ways infection are acquired by camels in their arid environment, its economic impact, as well as its public health significance, especially among the nomads who consume cameline milk and raw liver.

**Key words:** *ELISA, Camel, Sero-prevalence, Toxoplasma gondii.*

## INTRODUCTION

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that is probably capable of infecting all species of mammals, including man and cause one of the important zoonotic parasite diseases; Toxoplasmosis of world-wide distribution (Shaapan *et al.*, 2010). It can also cause abortion or neonatal mortality in other animal species, such as sheep, goats, horses, camels and deer (Dubey, 2003). Approximately one-third of humanity has been exposed to the parasite world wide (Evengard *et al.*, 1999; Sukthana, 2006). The parasite induces high levels of gamma interferon (IFN- $\gamma$ ) during initial infection as a result of early T-cell as well as natural killer (NK) cell activation. During chronic infection, parasite-specific T lymphocytes release high levels of IFN- $\gamma$ , which is required to prevent cyst reactivation (Denkers and Gazinelli, 1998). One attenuated vaccine has been successfully used to reduce abortions in sheep (Buxton, 1993); this live vaccine is unlikely to be used in humans. In addition; many inactivated vaccines developed in the past have produced only little to moderate protective efficacy against infections with a lethal dose of the virulent strain of *T. gondii*, therefore; effective and reliable vaccines comprising subunit or recombinant antigens are likely to be approved for use (Waldeland and Frenkel, 1986). Cats, including all felines; are definitive hosts for *T. gondii* and excrete environmentally-resistant oocysts in their feces (Dubey, 2006).

Domestic cats are probably the major source of contamination, because they are common and produce large numbers of *T. gondii* oocysts (Dubey and Frenkel, 1972; Dubey, 2001). *T. gondii* infection results in significant reproductive losses and economic losses (Faria *et al.*, 2007). Hosts became infected by ingestion of food or drink contaminated with oocysts or by ingesting undercooked meat from infected animals with *T. gondii* which causes mental retardation and loss of vision in congenitally- infected children and abortion in pregnant women and animals (Dubey *et al.*, 2005). Cats are essential in perpetuation of the life cycle, because *T. gondii* infection is rare or absent in areas devoid of cats (Wallace, 1969; Dubey *et al.*, 1997).

Seroprevalence data for cats are more useful than results of fecal examination, because cats with antibodies have probably already shed oocysts and are an indicator of environmental contamination (Dubey and Frenkel, 1972). Sporulated oocysts of *Toxoplasma gondii* are very resistant to environmental conditions. They remain infectious in moist soil or sand for up to 18 months (Frenkel, 2000). Serologic testing is an important method for detecting these parasitic infections, and includes immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), competitive-inhibition ELISA, Western blotting, and direct agglutination test (DAT) using intact tachyzoite or tachyzoitederived antigens (Jenkins *et al.*, 2002; Huang *et al.*, 2004), however; seroprevalence rates do vary depending on the serologic test and the initial serum dilution tested (Dubey, 2003).

Camels constitute an important economical activity in the desert population in east and central regions of Saudia Arabia. The camel is used as a load animal, which is an important role in transportation in rural areas; in addition, it is also a source of meat, skin, and leathers (Sadrebazzaz *et al.*, 2006).

Since there is a little information on the role of toxoplasmosis in camel in KSA; the presented study was aimed to estimate the seroprevalence of the *T. gondii* antibodies among camel (*Camelus dromedarius*) population in five provinces of Saudi, and subsequently to estimate the role of this parasite in the abortion of camel (*Camelus dromedarius*) as preliminary step in long lasting program to prevent transmit the infection to animal or man population due to its medical importance of *T. gondii* as a major source of parasitic zoonosis

## MATERIALS and METHODS

### 1. Blood samples

Random blood samples were collected from different farms with previous history of abortion and reproductive disorders from five provinces of KSA (Al-Riyadh, Alharig, Al- Solyl, Dar- maa and Wady Al- Dawaser): a total of 482 samples were collected (182 male and 300 female) from camels aged 3-6 years, by jugular vein-puncture and removed from the clotted blood by centrifugation at 3000 rpm for 15 minutes. All the sera were aspirated and kept in micro-tubes and stored at -20°C until tested for antibodies to *T. gondii*.

### 2. Enzyme linked immunosorbent assay (ELISA):

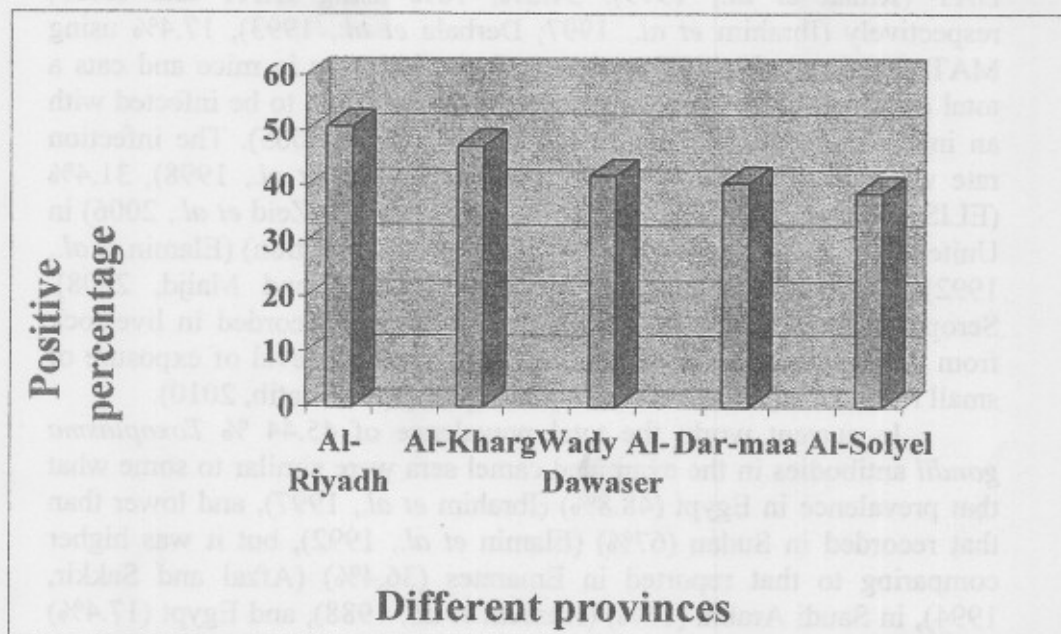
The commercial ELISA kits were supplied by Institute of Pourquier, France [Lot batch No., 2-TXRM- 003]. The test was carried out according to the manufacture's instructions. Each sample; individually tested in single well. The results were monitoring using ELISA reader with 450nm filter and expressed as the percentage of the mean absorbance value of the samples (S) to the mean absorbance value of the positive (P) control sample provided with the diagnostic kit. The resultant S/P ratio was expressed as a percentage (S/P %) according to manufacture's recommendation where; sera with S/P% less than 40% were regarded as negative, between 40% - 50% as suspicious, and more than 50% as positive.

## RESULTS

As shown in Table 1 the prevalence of *T. gondii* antibodies in examined camel sera revealed that 219 (45.44%) {42.86%male, 47% female} serum samples from totally examined 482 camels tested by ELISA were positive. Among which; 75(50.68%) {46.16% male,53.13% female}, 58(47.15%) {44.19% male and 48.75%} and 41(41.84%) {34.09% male and female 48.15%} had antibodies against *T gondii* in Al\_Riyadh., Alharig and Wady Al Dawaser respectively. But the prevalence is 28(40.58%) {38.71% male and 42.11% female}, 17(38.64%) {33.33% male and 40.63% female} in Dar-maa and Al Solyel.

**Table 1:** The prevalence (antibodies) of *Toxoplasma gondii* infection from five Province in Saudi Arabia.

Province	No. of examined animals			No. of positive animals			% of positive		
	♂	♀	Total	♂	♀	Total	♂	♀	Total
Al-Riyadh	52	96	148	24	51	75	46.16	53.13	50.68
Al_Kharg	43	80	123	19	39	58	44.19	48.75	47.15
Wady Al Dawaser	44	54	98	15	26	41	34.09	48.15	41.84
Dar-maa	31	38	69	12	16	28	38.71	42.11	40.58
Al Solyel	12	32	44	4	13	17	33.33	40.63	38.64
Total	182	300	482	78	141	219	42.86	47.00	45.44



**Fig. 1:** Percentage of positive cases in different provinces

## DISCUSSION

*T.gondii* is a protozoan parasite that infects mainly the cat as a definitive host and sheep as a very important intermediate host. In ewes, this parasite is now considered to be the major cause of abortion worldwide (Dubey and Beattie, 1988); it has been found worldwide from Alaska to Australia so public health organizations repeatedly encourage the collection of accurate data about *T. gondii* in animals and humans due to its medical importance as a major source of parasitic zoonosis (Rinaldi and Scala, 2008). In KSA, there role of toxoplasmosis were recorded as one of the most important causative agent of abortion and infertility in both sheep and goat (Al-Khatib, 2010).

Toxoplasmosis usually results from the ingestion of food contaminated with oocysts or cysts of the parasite. Oocysts are shed by cats -the definitive hosts of *Toxoplasma gondii* - while; cysts are present in tissues from chronically infected animals (Dubey and Beattie, 1988). The prevalence data for the determination of *T. gondii* infection in camels at the different localities of the world is extremely variable. In Egypt, the infection rates among slaughtered camels were 46% using DAT (Rifaat *et al.*, 1979), 54.2%, 18% using IHAT and IFAT, respectively (Ibrahim *et al.*, 1997; Derbala *et al.*, 1993), 17.4% using MAT (Hilali *et al.*, 1998) and recently by bioassay in mice and cats a total of 23 out of 90 camel meat samples were found to be infected with an incidence rate of 25.6% (Shaapan and Fathia, 2005). The infection rate was 16% (IHAT) in Saudi Arabia, (Hussein *et al.*, 1998), 31.4% (ELISA) (Abu-Zeid, 2002) and 22.4% (MAT) (Abu-Zeid *et al.*, 2006) in United Arab Emirates; in Sudan 67% (latex agglutination) (Elamin *et al.*, 1992) and 51.3% (latex agglutination) (Manal and Maijd, 2008). Seroprevalence data on *T. gondii* that have been recorded in livestock from different regions in Saudia, showing the high level of exposure of small ruminants (76.84% for sheep and goats) (Al-Khatib, 2010).

In current work; the total prevalence of 45.44 % *Toxoplasma gondii* antibodies in the examined camel sera were similar to some what that prevalence in Egypt (48.8%) (Ibrahim *et al.*, 1997), and lower than that recorded in Sudan (67%) (Elamin *et al.*, 1992), but it was higher comparing to that reported in Emarates (36.4%) (Afzal and Sakkir, 1994), in Saudi Arabia (16%) (Hussein *et al.*, 1988), and Egypt (17.4%) (Hilalia *et al.*, 1998) among camel population. The variety of prevalence of toxoplasmosis in the current and previous studies could emanate from

difference in the serological tests employed and the initial serum dilution tested (Dubey, 2003), geographical variability or continuous exposure of camels to infection due to heavy environmental contamination with oocysts shed from the observed stray cats in the farms, frequency of felines on the farms, age of the animals, and the climatic variations from one region to another (Dubey, 1990; Sawadogo *et al.*, 2005). Variation in prevalence of *T. gondii* occurs not only among different continents or countries but also within a given country (Teshale *et al.*, 2007), camels free range livestock associated with *T. gondii* infection, acquisition of Toxoplasma infection by camels is through ingestion or inhalation of sporulated oocysts that are shed by cats in the environment, they are kept on pastures with an increased pressure of infection due to contamination of environment with oocysts and the frequency of stray cats in a humid rainy climate. The high ratio of *Toxoplasma gondii* obtained in these work may be attributed also to the fact that, the stray cats are widely spread specially in El-Rhyaid which is in favor of a higher prevalence of oocysts within hot and humid environments compared to cold and dry ones is attributed to the longer viability of *T. gondii* oocysts under humid conditions (Fleck, 1972; Fayer, 1981; Elamin *et al.*, 1992; Remington *et al.*, 2001). The effect of sex on the prevalence of *T.gondii* in camel obtained in our study indicated that infection rates were higher in female than male; in this regard; (Dubey and Beattie 1988; Randall *et al.*, 2000) proved that antibody prevalence of *T. gondii* was directly related to sex and they ascribed this fact to opportunity stresses (pregnancy and lactation) exposed by females. This study suggests widespread infection with *Toxoplasma gondii* among camels, a finding that warrants a closer look into the possible ways infection are acquired by camels in their arid environment, its economic impact, as well as its public health significance, especially among the nomads who consume cameline milk and raw liver.

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