Toxoplasma gondii AND *Rotavirus* ASSOCIATED WITH CAMEL-CALF DIARRHEA IN SUDAN

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Abstract

The aim of this study was to determine the role of *Toxolasma gondii* and rotavirus infection in camel-calves diarrhea in Sudan. *Toxoplasma* gondii and Rotavirus are considered as causative agents of calf-diarrhea. 278 serum samples were collected from diarrheic camel-calf (less than one year age) in five locations in Sudan ; River Nile(North), El-Gedarif (East), Sennar &Blue Nile (Central to South) and kordofan (West). Out of 278 serum samples,157 sera (56.5%) were sero-positive for anti-*Toxoplasma* antibodies by latex agglutination test, ELISA test was applied on the sero-reacted sera, IgM and IgG were detected in sera. Also a competitive ELISA kits for rotavirus antibodies detection were used , results showed that 66 sera (23.7%) were sero-positive for rotavirus antibodies . Statistical analysis using software analysis programs showed no significant difference (P>.05) between the five surveyed locations for both *Toxoplasma* and rotavirus infections. The sero-prevalence of rotavirus in different age groups was found to be statistically significant (P<.05), however, there is no statistical difference in the occurrence of *Toxoplasma* in different age groups ; this may reveal an occurrence of congenital infection. There was no statistical difference (P >.05) in the occurrence of sero-prevalence of *Toxoplasma* and rotavirus in males and females.

المستخلص

تهدف هذه الدراسة تحديد دور عدوى طفيل التوكسوبلازما و فيروس الروتا في الاصابة باسهالات عجول الجمال في السودان حيث تعتبر التوكسوبلازما و فيروس الروتا من العوامل المسببة للاسهال فى عجول الجمال. تم جمع 278 عينة مصل من الإبل المصابة بالاسهال (أقل من عمر سنة واحدة)و ذلك من خمسة مواقع بالسودان وهى؛ نهر النيل، القضارف ، سنار, النيل الأزرق وكردفان ، اظهرت النتائج بأن 157 عينة مصل (56.5٪)كانت ايجابية للأجسام المناعية المضادة للتوكسوبلازما بواسطة اختبار تراص اللاتكس كما تم تاكيد النتائج الايجابية عن الأجسام المناعية المضادة للتوكسوبلازما باجراء اختبار ELISA .

تم اجراء اختبار ELISA للكشف عن الأجسام المضادة التنافسية لفيروس الروتا . وأظهرت النتائج أن عدد 66 من الأمصال (23.7٪) مصابة بالفيروس . أظهر التحليل الإحصائي باستخدام برامج تحليل البرامج انه لا يوجد فرق كبير بين المواقع الخمسة التي شملتها الدراسة (p<0.5)

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لكل من عدوى التوكسوبلازما و فيروس الروتا . وقد وجد أن معدل انتشار فيروس الروتا بين الفئات العمرية ذو فروقات معنوية $p>0.5$ لتكون ذات دلالة إحصائية, و لكن، لا يوجد فرق إحصائي في وقوع التوكسوبلازما في مختلف الفئات العمرية $p<0.5$, هذا قد يكشف عن حدوث العدوى الخلقية . لم يكن هناك فرق إحصائي للتوكسوبلازما وفيروس الروتا بين مجموعات الذكور والإناث $p<0.5$.

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Introduction

High mortality of camel- calves is considered one of the major problems to higher productivity in camels. Many factors contribute to calves mortality, among which is calf diarrhea (Schwartz,1992). Neonatal calf diarrhea is a costly disease. Cases of calf diarrhea were reported in camel herds in Sudan giving a prevalence of 83% with a mortality of 39.9% (Ali, *et al*.,2005) . In eastern Sudan, camel- calf diarrhea affects about 33% of the neonates causing a death loss of up to 23% (Abbas, *et al*., 1992) .

There are numerous infectious causes of calf diarrhea, which may be present either singly or in combination. *Toxoplasma gondii* and *Rotavirus* are considered as causative agents of camel-calf diarrhea in Sudan . (Manal, *et al*. (2005 b) reported that, diarrhea was the main clinical sign that appeared on camel –calves which were delivered from mothers experimentally infected with *Toxoplasma* sporocysts during pregnancy. On the other hand, Rotavirus was detected by ELISA in diarrheic camel-calves in eastern Sudan (Mohamed, *et al*.,1998 ; Ali, *et a*l.,2003) . Many reports revealed a wide-spread prevalence of anti-*Toxoplasma* antibodies among Sudanese camels locations, the last attempt revealed an overall prevalence of 61.7% in six different locations (Manal, *et.al*.,2005).

Materials and Methods

Samples collection : A total of 278 blood samples were obtained in plain vacutainers from diarrheic camel calves less than 12 months of age- owned by nomads from different locations in Sudan; 43 sera from River Nile (North) , 41 sera from El-Gedarif (East) , 21 sera from Sennar and Blue Nile (Central to South) and 172 sera from Kordofan (West) . Blood samples were left to clot overnight at 4^{o}C and sera was decanted into plastic tubes and stored at -20^{o}C until used.

Detection of anti-*Toxoplasma* antibodies : Latex agglutination test was applied on the collected sera using Toxo-latex kit(Linear chemicals , SL,Spain) . ELISA test was applied on the sero-reacted sera; IgM and IgG ELISA kits (Linear Kemecals Copm. Spain), the test was applied according to the manufacturer guides .

Sero-epidemiology of *Rotavirus* in camels : A competitive ELISA kits for rotavirus antibodies detection (Bio x diagnostics-Belgium) were used . The test was performed according to the manufacturer instructions.

Results

Results showed that, out of 278 serum samples collected from different states, 157 sera (56.5 %) were found to be positive for *Toxolasma* anti- antibodies and 66 sera (23.7%) were positive for *Rotavirus* antibodies (Table 1), the distribution of these figures in the four tested areas is shown in Figure 1. The results showed that

,there was no significant difference (P-value>.05) in the occurrence of sero-prevalence of *Toxoplasma* and *Rotavirus* in the different location .

Sex distribution of *Toxoplasma* and *Rotavirus* seropositive camels:

The detected seropositives of *Toxoplasma* in camel calves were 52.8% in females and 61.4 % in

males while the detected *Rotavirus* antibodies were 22.9% in females and 24.6% in males. The difference in the occurrence of sero-prevalence of rota and *Toxoplasma* in females and males was found to be statistically insignificant (P-value>.05). (Fig.3) .

Age distribution of *Toxoplasma* and *Rotavirus* seropositive camels:

It was noticed that most of positive samples for *Toxoplasma* were at less than 3 months of age (58.8%) while the highest percentage of positives for *Rotavirus* were at more than 9 months and less than 18 months of age (Figure 2). Calves aged between nine to twelve months were showed a highest percentage of infection with both *Toxoplasma* and rotavirus (30.4%) while calves under three months of age showed a percentage of 11.3% (Fig. 4).

Distribution of *Toxoplasma* and *Rotavirus* co-infection:

The highest percentage of *Toxoplasma* and *Rotavirus* co-infection was noticed in samples collected from eastern Sudan (Gedarif) then Northern Sudan (River Nile), the details are presented in Figure 3

Titers of *Toxoplasma* and *Rotavirus* antibodies:

Different titers of antibodies were detected in tested camel calf sera, highest titer of *Toxoplasma* (1/1024) was seen in 17.3% of samples and 1/512 was detected in 21.2% of samples (Figure 5).

Highest *Rotavirus* antibodies titer (+4) was noticed in only 15.1% of samples while lowest titer (+1) was seen in 34.8% of sera (Figure 6).

Discussion

This study focused on *Toxoplasma* which was previously reported as one of the causative agents of camel-calf diarrhea (Manal, *et al*., 2008) and also on *Rotavirus* which is known to be the main cause of diarrhea in young animals. The detected *Toxoplasma* antibody was 56.5% of 278 sera which is considered to be high as shown in a previous study that revealed detection of anti-*Toxoplasma* antibodies in 51.3% of samples collected from different parts of Sudan with more or less the same prevalence rate, indicating the widespread of *Toxoplasma* infection among camel-calf (Manal, *et al*.,2008).

Previous reports described the detection of *Rotavirus* antibodies in camels, Mahin *et al* (1983) detected Rotavirus antibodies in 27 of 55 camel calves in Morocco. El Sayed, *et al* (1992) reported the presence of high titer of *Rotavirus* antibodies in

camel milk. In a recent study in Sudan *Rotavirus* antibodies were detected in 48% of tested camel calf sera (Ali, *et al*., 2005).

In this study *Rotavirus* antibodies were detected in 23.7% of 278 tested camel calf sera aged 1-17 months, this seroprevalence is far lower than that detected in the previous study (48%) reported by Ali, *et al.* (2005), this is mainly due to the age group tested which was 1-17 months while in the previous study most of samples were collected from camels aged 18-36 months; at this age several exposures to the virus are expected building high immunity. No significance difference was seen between *Rotavirus* prevalence in males and females.

In this study we focused on co-infection of *Toxoplasma* and *Rotavirus*, about 60% of sera showed evidence of co-infection. Most of detected co-infection was in 9-17 month of age.

The age group 1-3 month showed the highest percentage of positives for *Toxoplasma* and the lowest percentages for *Rotavirus*; this is due to the presence of *Toxoplasma* antibodies which are usually found in the newborn calves due to the congenital infection, the same findings were previously reported by Manal et al (2008), while *Rotavirus* antibodies came later due to the exposure of calves to the virus.

It was noticed that, the highest percentage of *Toxoplsma* and *Rotavirus* co-infection was detected in sera collected from Gedarif state. This is expected as this area (Butana) is usually being a grazing and watering area for camels during autumn, which leads to the persistance and distribution of the infection.

In the present study a highest titer of *Toxoplasma* was seen in 38.5% of sera , while a highest titer of *Rotvirus* was observed in 34.9% of sera . This indicates the significant role of the two pathogens in causing diarrhea .

Table 1: Detection of *Toxoplasma gondii* and *Rotavirus* antibodies in camel sera in Sudan using ELISA

Antibodies	Total tested sera	No. of positive sera	No. of negative sera	Percentage of positive sera
Toxoplasma	278	157	121	56.5
Rotavirus	278	66	212	23.7

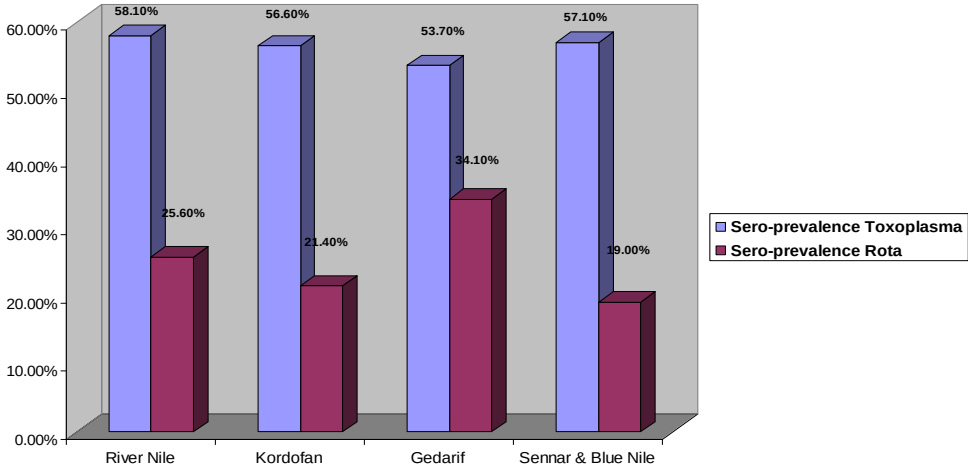

Figure 1: Seroprvalence of *Toxoplasma* and *Rotavirus* in camels in four states of Sudan.

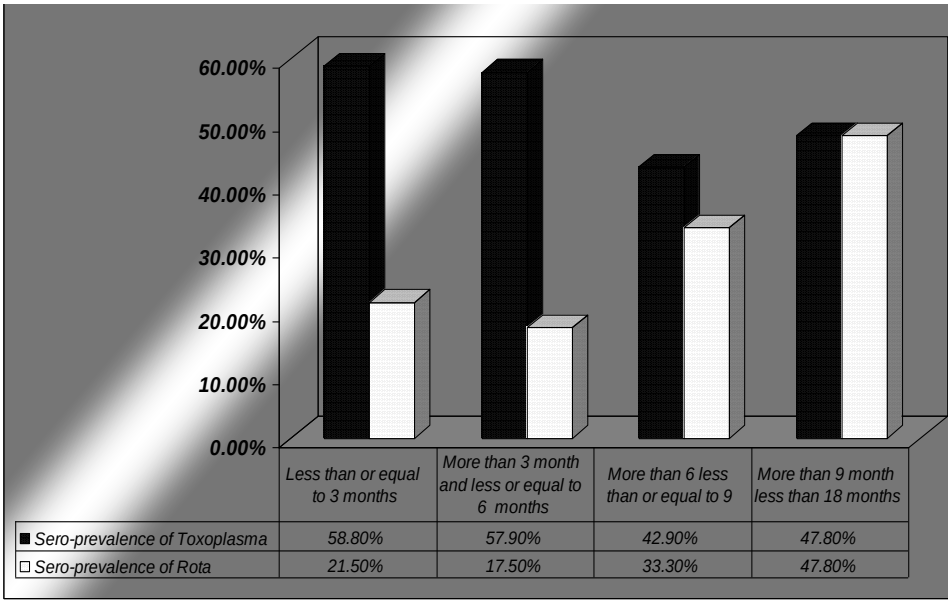

Figure 2: Seroprevalenc of *Toxolasma* and *Rotavirus* in different age groups

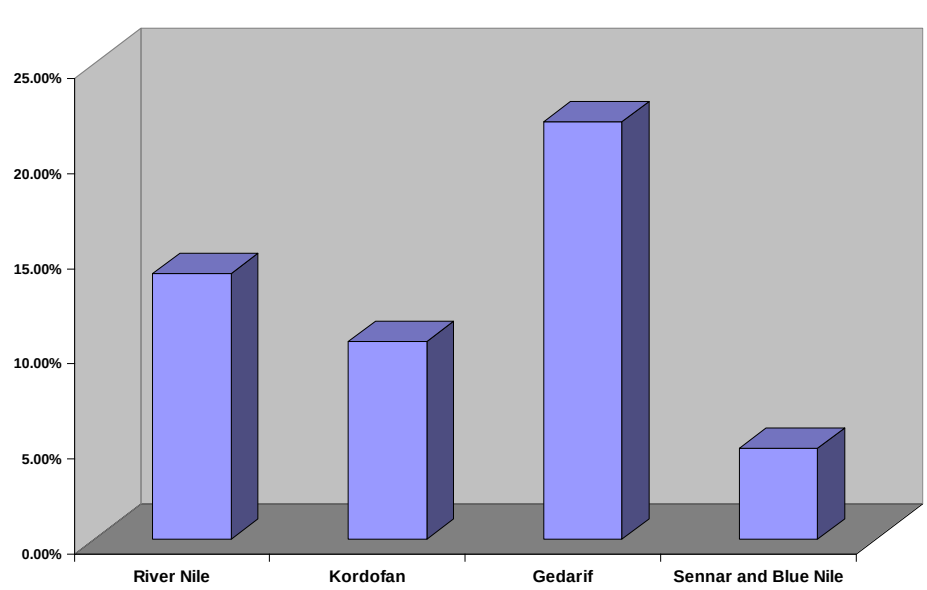

Figure 3: Percentage of samples positive to both *Toxoplasma* and *Rotavirus* in different states

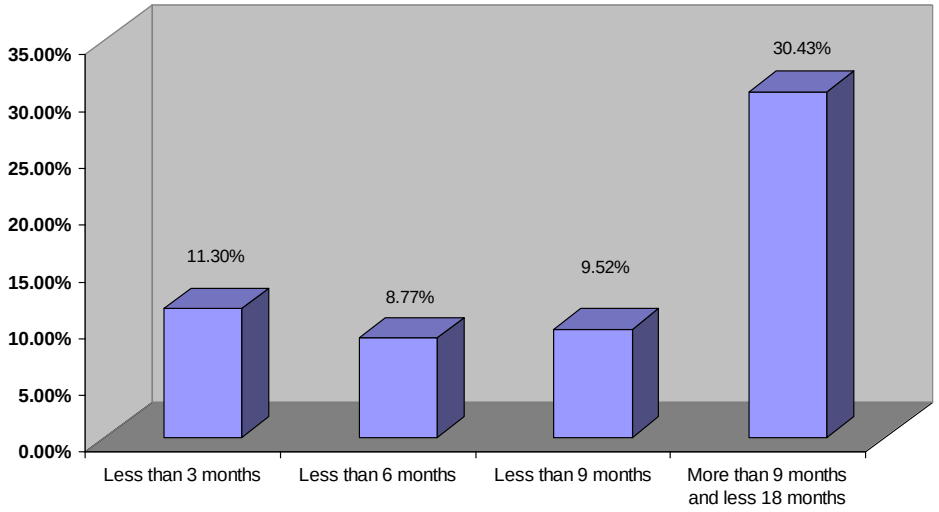

Figure 4: Percentage of animals with *Toxoplasma* - *Rotavirus* co-infection

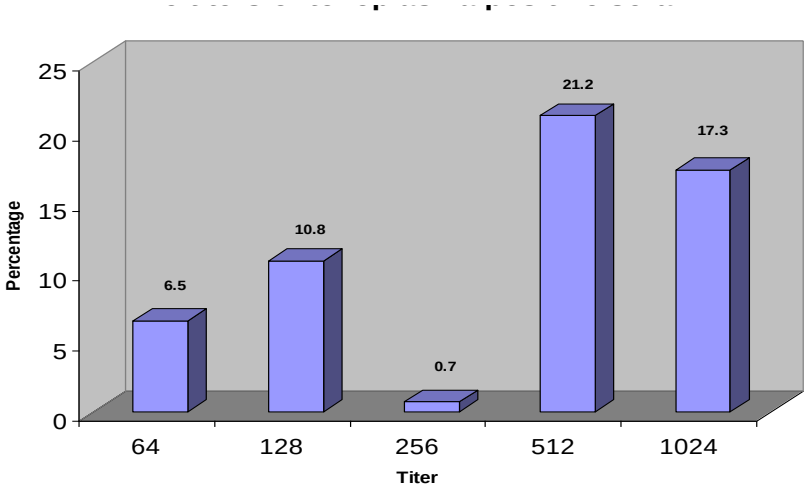

Figure 5: The titer of positive *Toxoplasma* antibodies in camel sera

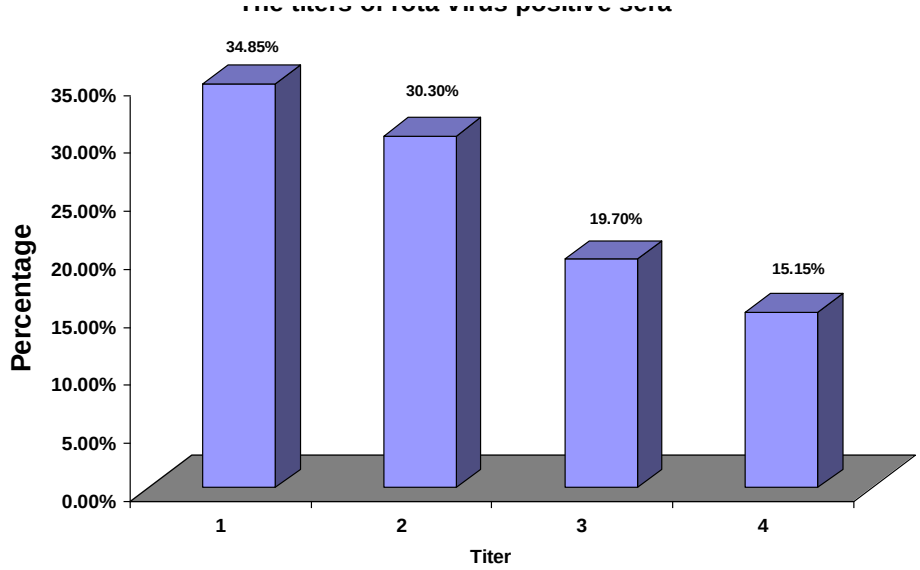

Figure 6: The titer of positive antibodies Rotavirus in camel sera

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