Studies on *Toxoplasma* and *Sarcocystis* from Camels (*Camelus dromedaries*) in the Sudan

By
Manal Yousif Ishag
B.V.Sc.(1987)University of Zagazig
M.V.Sc.(1996)University of Khartoum

Supervisor:
Professor Mohamed Magzoub Alkan
Co-Supervisor:
Professor Ali Mohamed Abd ElMajid

A thesis submitted to the Graduate College of the University of Khartoum in fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Parasitology
Faculty of Veterinary Medicine
University of Khartoum

May 2003
PREFACE

The study was conducted under the supervision of Professor Mohamed Magzoub Alkan and the Co-supervision of Professor Ali Abdel Majid.

The work of this thesis was carried out in the Department of Pathology and Diagnosis, Central Veterinary Research Laboratories, Animal Resources Research Corporation.
DEDICATION

To my parents, husband

and

To my beloved children,

Hussam, Awab, Montaha

and

Raian
ACKNOWLEDGEMENTS

First of all my thanks to Allah for giving me health and strength to carry out this work.

I am feeling very grateful to my supervisor, Professor, Mohamed Magzoub Alkan for his invaluable help and assistance during the study and preparation of this thesis.

I am deeply indebted to my co-supervisor, Professor, Ali Abd El Majid for the facilities and financial support for providing me with the experimental camels and sero-diagnostic kits, and for his invaluable guidance and encouragement.

Thanks are due to Professor Siddig El Aowni

I am also deeply indebted to Dr. Yahia H. Ali for his unfeeling help during collection of camel sera. My gratitude is also extended to Dr. Elzein Basheer for his help in computer data analysis.

I extend my gratitude to the staff of the Department of Diagnosis and Pathology, Central Veterinary Research Laboratories for their cooperation and help during my studies.

Special thanks for Mr. Farooq Idris and Mr. Abdel Raheem Seed Ahmed for their assistance and help with camels and collection of samples. Thanks are also extended to Mr. Yousif Abd Elwahab for his assistance during preparing histo-pathology sections.

Finally, I am grateful to Mrs. El Toma Mohamed for typing this thesis and Mr. Mohamed El Mustafa for photography.
الكير.. длин.. المجموعة.. العمر.. الفترة.. الشمال.. اختبار.. الزمان.. الأجنب.. 6 إلى الحمل أول.. الناقة.. التي.. بعده.. 10%.. كمية.. وتBLEM.. الحمل.. النازعة.. كمية.. 0.51.. X.. اختبارها.. و.. الحمل.. النازعة.. أثناء.. على.. الحولة.. الشخصية.. في.. (النقاء.. في..، كمية.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. ل.. في.. 10%.. مح.. في..،.. في.. 51.. يق.. أن.. الهر.. أربعة.. على.. أنا..،.. في.. 51.. التاريخ.. يحدث.. في.. 1024:1.. أن.. كمية.. الجسم.. 61.7%.. السيدة.. بالتكسب.. L
لا يوجد نص يمكن قراءته بشكل طبيعي من الصورة المقدمة.
abetic Vox Lux 70 مصداقية و 1424204596 وأervation 10 × 10 موم 2410212 12624295 و 142291944345 و 142291944345 و 1424204596 وأervation 10 × 10 موم
ABSTRACT

In this study *Toxoplasma gondii* antibodies were detected in six locations of camels in the Sudan, using latex agglutination test. The overall prevalence was 61.7%; 68% in El Gedarif, 60% in North Kordofan, 57% in El Shawak, 51% in River Nile, 51% in Butana and 43% in El Hamra. A highly significant difference between the seroprevalence in different age groups was established (P<0.01). This result revealed a high prevalence among old animals. Camels under one year of age have a high titer at 1:1024, the other age groups showed titers ranged between 1:512 and 1:128. There were no significant sex-linked differences in the different sex groups.

*Toxoplasma gondii* oocysts were isolated from kittens that were fed raw cameline meat. Four pregnant she-camels at different stage of pregnancy and serologically negative for *Toxoplasma gondii* were infected with different doses of infective *T. gondii* oocysts (1x10^5 or 1x10^6 sporocysts). This study showed that the outcome of *T. gondii* infection varied according to the time of infection during pregnancy and the number of parasites inoculated; she-camel infected with 1x10^6 infective *T. gondii* oocysts during the first half of pregnancy, delivered dead calf-camel, she-camel infected with 1x10^5 infective *T. gondii* oocysts during the first half of pregnancy delivered a weak calf-camel, which showed corneal opacity and survived for 12 days, she-camel infected with 1x10^5 *T. gondii* oocysts at second half of pregnancy delivered a calf-camel that appeared normal at birth but it was unable to stand and needed assistance in suckling, it died at day 40 of age, she
camel infected with $1 \times 10^6$ *T. gondii* oocysts at the second half of pregnancy delivered very weak, refused suckling and had diarrhoea. *Toxoplasma* tachyzoites and cysts were found in all infected calf-camels and in all infected mice. All calf-camels showed diarrhoea, gross examination revealed enlargement of lymph nodes, haemorrhages in brain, lung, kidneys, eyes and skeletal muscles. The main lesions detected microscopically in the calf-camels were haemorrhages, infiltration of lymphoid cells and congestion in lung, liver, brain, intestine, kidney and heart. Liver showed necrosis, lung showed oedema and emphyzema. Intestines of the calf-camels showed severe damage of epithelial cells, haemorrhage, congestion of blood vessels, infiltration of lymphocystes (mainly eosenophils) and aggregation of *Toxoplasma* tachyzoites. Focal necrosis and haemorrhages were detected in placentae of all infected she-camels. The placentae were enlarged and fragile. Microscopic findings revealed focal necrosis, infiltration of mononuclear cells and haemorrhages.

The excretion of *Toxoplasma gondii* in milk of camels was studied by inoculation of mice with milk of three experimentally infected lactating she-camels. *Toxoplasma* tachyzoites and cysts were detected in the brain of the suckling calf-camels and mice. *Toxoplasma* antibodies were detected in sera of all infected suckling calf-camels with low antibody titers. This may reveal that the concentration of *Toxoplasma* tachyzoites in the milk of camels was low.
Sarcocystis sporocysts were isolated from intestinal mucosae of puppies that had been fed on raw cameline meat. Calf-camels infected orally with different doses of Sarcocystis sporocysts showed different gravity of infection; calf-camel inoculated with $1 \times 10^6$ and $1 \times 10^5$ S. sporocysts died 21 days after infection and 27 days after infection respectively. Microscopic examination of tissues revealed haemorrhages, oedema and infiltration of lymphoid cells in lung, liver showed vacuolation of cells and haemorrhages. Haemorrhages were detected in heart, lymph nodes, spleen and intestine. Schizonts were detected within endothelial cells of blood vessels of oesophagus, diaphragm and tongue. Calf-camel infected with $1 \times 10^2$ S. sporocysts died 90 days after infection. Microscopic examination revealed the presence of Sarcocystis cysts located lengthwise between the muscle fibers of the heart, oesophagus, diaphragm, tongue and skeletal muscles.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Acknowledgements</th>
<th>i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabic کرایت</td>
<td>ii</td>
</tr>
<tr>
<td>Abstract</td>
<td>iv</td>
</tr>
<tr>
<td>List of tables</td>
<td>x</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xi</td>
</tr>
</tbody>
</table>

## CHAPTER I .................................

1. Introduction and literature review ....... 1
1.1. Introductory remarks........................ 1
1.2. Taxonomy..................................... 3
1.3. Morphology................................... 4
1.3.1 *Toxoplasma*................................ 4
1.3.2 *Sarcocystis*................................ 5
1.4. Life cycle.................................... 5
1.4.1 *Toxoplasma*................................ 5
1.4.2 *Sarcocystis*................................ 7
1.5. *Toxoplasma* and *Sarcocystis* species from camels............................... 8
1.6. Transmission.................................... 9
1.6.1 *Toxoplasma*................................ 9
1.6.2 *Sarcocystis*................................ 9
1.7. Pathogenicity.................................. 9
1.7.1. *Toxoplasma*................................. 9
1.7.2. *Sarcocystis*................................. 11
1.8. Survival of sporocysts....................... 12
1.8.1 *Toxoplasma*................................. 12
1.8.2. *Sarcocystis*................................. 13
1.9. Survival of tissue-cysts..................... 13
1.9.1 *Toxoplasma*................................. 13
1.9.2 *Sarcocystis*................................. 14
1.10. Diagnosis..................................... 14
1.10.1 *Toxoplasma*................................. 14
1.10.2 *Sarcocystis*................................. 15
## CHAPTER II

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. General Materials and Methods</td>
<td>17</td>
</tr>
<tr>
<td>2.1 Detection of <em>Toxoplasma</em> antibodies in Sudanese Camels</td>
<td>17</td>
</tr>
<tr>
<td>2.2 Experimental animals</td>
<td>18</td>
</tr>
<tr>
<td>2.2.1 Dogs and Cats</td>
<td>18</td>
</tr>
<tr>
<td>2.2.2 Camels</td>
<td>18</td>
</tr>
<tr>
<td>2.3 Preparation of meat samples</td>
<td>19</td>
</tr>
<tr>
<td>2.4 Experimental infection of dogs and cats</td>
<td>20</td>
</tr>
<tr>
<td>2.5 Detection of oocysts and/or sporocysts in faeces of dogs and cats</td>
<td>20</td>
</tr>
<tr>
<td>2.6 Sporulation of oocysts voided by experimental animals</td>
<td>21</td>
</tr>
<tr>
<td>2.7 Isolation of <em>Toxoplasma</em> oocysts</td>
<td>21</td>
</tr>
<tr>
<td>2.8 Preparation of <em>Sarcocystis</em> inoculum</td>
<td>22</td>
</tr>
<tr>
<td>2.9 Experimental infection of camels with <em>Toxoplasma gondii</em> oocysts</td>
<td>23</td>
</tr>
<tr>
<td>2.10 Experimental infection of calf-camels with <em>Sarcocystis sporocysts</em></td>
<td>23</td>
</tr>
<tr>
<td>2.11 Detection of <em>Toxoplasma</em> antibodies</td>
<td>23</td>
</tr>
<tr>
<td>2.12 Histological Methods</td>
<td>24</td>
</tr>
<tr>
<td>2.13 Examination of products of conception for <em>Toxoplasma</em></td>
<td>24</td>
</tr>
<tr>
<td>2.14 Examination of mice for <em>Toxoplasma</em> infection</td>
<td>25</td>
</tr>
<tr>
<td>2.15 Examination of calf-camels tissues for encysted <em>Toxoplasma gondii</em></td>
<td>25</td>
</tr>
<tr>
<td>2.16 Haematological methods</td>
<td>25</td>
</tr>
<tr>
<td>2.16.1</td>
<td>Erythrocyte and leukocyte counts</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>2.16.2</td>
<td>Estimation of haemoglobin concentration</td>
</tr>
<tr>
<td>2.16.3</td>
<td>Determination of packed cell volume</td>
</tr>
<tr>
<td>2.16.4</td>
<td>Differential count of white blood cells</td>
</tr>
<tr>
<td>2.17</td>
<td>Examination of milk for <em>Toxoplasma gondii</em></td>
</tr>
</tbody>
</table>

### CHAPTER III

3. Sero-prevalence of *Toxoplasma gondii* antibodies in Sudanese camels

<table>
<thead>
<tr>
<th>3.1.</th>
<th>Introduction</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.</td>
<td>Materials and Methods</td>
<td>28</td>
</tr>
<tr>
<td>3.3.</td>
<td>Results</td>
<td>29</td>
</tr>
<tr>
<td>3.4.</td>
<td>Discussion</td>
<td>32</td>
</tr>
</tbody>
</table>

### CHAPTER IV

4. Congenital Toxoplasmosis of camels

<table>
<thead>
<tr>
<th>4.1.</th>
<th>Introduction</th>
<th>44</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.</td>
<td>Materials and Methods</td>
<td>45</td>
</tr>
<tr>
<td>4.3.</td>
<td>Results</td>
<td>46</td>
</tr>
<tr>
<td>4.4.</td>
<td>Discussion</td>
<td>65</td>
</tr>
</tbody>
</table>

### CHAPTER V

5. Detection of *Toxoplasma gondii* tachyzoites excreted in milk of experimentally infected camels

<table>
<thead>
<tr>
<th>5.1.</th>
<th>Introduction</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2.</td>
<td>Materials and Methods</td>
<td>70</td>
</tr>
<tr>
<td>5.3.</td>
<td>Results</td>
<td>71</td>
</tr>
<tr>
<td>5.4.</td>
<td>Discussion</td>
<td>72</td>
</tr>
</tbody>
</table>
CHAPTER VI

6. The effect of acute Sarcocystosis on camels....... 75
   6.1. Introduction......................................... 75
   6.2. Materials and Methods........................... 76
   6.3. Results............................................. 77
   6.4. Discussion......................................... 84

CHAPTER VII

7. General Discussion..................................... 88
REFERENCES............................................ 96
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sero-prevalence of <em>Toxoplasma</em> in the different locations from which the samples were collected</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>Sero-prevalence of <em>Toxoplasma</em> in the Butana, North Kordofan and River Nile areas</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Sero-prevalence of <em>Toxoplasma</em> in the different age groups</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>The titers frequency of <em>Toxoplasma</em> for the positive animals in different age groups</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>Sero-prevalence of <em>Toxoplasma</em> in the different sex groups in Butana area</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>Sero-prevalence of <em>Toxoplasma</em> in the different sex groups in North Kordofan area</td>
<td>39</td>
</tr>
<tr>
<td>7</td>
<td>Sero-prevalence of <em>Toxoplasma</em> in the different sex groups in River Nile area</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>Sero-prevalence of <em>Toxoplasma</em> in the different sex groups</td>
<td>41</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The distribution of sero-positivity in the serum samples..................</td>
<td>30</td>
</tr>
<tr>
<td>2.</td>
<td>The distribution of the sero-positivity in the sample after the sample area into large units.</td>
<td>34</td>
</tr>
<tr>
<td>3.</td>
<td>Frequency of the titer in the different age groups</td>
<td>37</td>
</tr>
<tr>
<td>4.</td>
<td>Calf-camel 1 at day of birth</td>
<td>48</td>
</tr>
<tr>
<td>5.</td>
<td>A histosection in the brain of calf-camel 1 (H &amp; E)x100.</td>
<td>50</td>
</tr>
<tr>
<td>6.</td>
<td>A histosection in the liver of calf-camel 1 (H &amp; E) x 100.</td>
<td>50</td>
</tr>
<tr>
<td>7.</td>
<td>A histosection in the lung of calf-camel 1 (H &amp; E)x100.</td>
<td>51</td>
</tr>
<tr>
<td>8.</td>
<td>A histosection in the kidney of calf-camel 1 (H &amp; E)x100.</td>
<td>51</td>
</tr>
<tr>
<td>9.</td>
<td>A photograph of calf-camel 2 at day of birth</td>
<td>52</td>
</tr>
<tr>
<td>10.</td>
<td>A histosection in the brain of calf-camel 2 (H &amp; E)x400.</td>
<td>54</td>
</tr>
<tr>
<td>11.</td>
<td>A histosection in liver of calf-camel 2 (H &amp; E)x100.</td>
<td>54</td>
</tr>
<tr>
<td>12.</td>
<td>A histosection in lung of calf-camel 2 (H &amp; E)x100.</td>
<td>55</td>
</tr>
<tr>
<td>13.</td>
<td>A histosection in brain of calf-camel 3 (H &amp; E)x100.</td>
<td>55</td>
</tr>
<tr>
<td>14.</td>
<td>A histosection in liver of calf-camel 3 (H &amp; E)x100.</td>
<td>56</td>
</tr>
<tr>
<td>15.</td>
<td>A histosection in lung of calf-camel 3 (H &amp; E)x100.</td>
<td>56</td>
</tr>
</tbody>
</table>
16. A histosection in kidney of calf-camel (H & E)x100. ........................................ 57
17. A photograph of calf- camel 4.................. 59
18. A photograph of calf-camel 4. .............. 60
19. A histosection in brain of calf-camel 4 (H & E)x100. ........................................ 61
20. A histosection in liver of calf-camel 4 (H & E)x100. ........................................ 61
21. A histosection in lung of calf-camel 4 (H & E)x100. ........................................ 62
22. A histosection in intestine of calf-camel (H & E)x100. ........................................ 62
23. A photomicrograph of Toxoplasma tachyzoites (H & E)x100....................... 63
24. A photograph of Toxoplasma gondii cyst in mouse brain (H & E)x400............. 64
25. A photograph of placenta. .................. 66
26. Toxoplasma antibody titers of she-camels and calf-camels at day of birth............. 67
27. A photomicrograph of Toxoplasma cysts in a brain of suckling calf-camel (H & E)x400. ...... 73
28. RBCs values of calf-camels infected with sarcocystis........................................ 78
29. Haemoglobin g/dl for calf-camels infected with Sarcocystis.............................. 79
30. PCV values of calf-camels infected with Sarcocystis................................. 80
31. A histosection in lung of calf-camel 1 (H & E)x100. ........................................ 81
32. A histosection in lymph node of calf-camel 1 (H & E)x100. .............................. 81
33. A histosection in lung of calf-camel II (H & E)x400. .......................................................... 83
34. A histosection in liver of calf-camel II (H & E)x100. ......................................................... 83
35. A photomicrograph of *Sarcocystis* cyst between muscle fibers of calf camel III (H & E)x400.......................................................... 85
36. A photomicrograph of immature *Sarcocystis* cyst in brain of calf-camel III (H & E)x400...... 85
37. A histosection in lung of calf-camel III (H & E)x100. .......................................................... 86
38. A histosection in liver of calf-camel III (H & E)x100. .......................................................... 86
CHAPTER I

Introduction and Literature Review

1. Introduction and Literature Review
1.1. Introductory remarks:

*Toxoplasma gondii* is a world-wide, polyxenous, intracellular coccidian parasite, has a facultative heteroxenous life cycle and can probably infect all warm-blooded animals (mammals, birds and humans). The first description of *Toxoplasma gondii* merozoites in the spleen, liver and blood of gondis, North African rodents, was given by Nicolle and Manceaux (1908). In 1965, Hutchison isolated *Toxoplasma* oocysts from the faeces of cats. Frenkel *et al.*, (1970); Dubey *et. al.* (1970) completed a knowledge of the coccidian life cycle of *Toxoplasma gondii* by the discovery of sexual stages in the small intestines of cats.

*Toxoplasma* infection among animals is of great importance, because some of the infected animals play a distinct role as the source of human infections. The infection in cats has the most important role in the epidemiology of toxoplasmosis, since infected cats excrete *Toxoplasma* oocysts which after sporulation become infectious to humans and animals and remain infectious for a long time. *Toxoplasma* cysts in the tissue of animals may serve as another source of human infection.

Toxoplasmosis is an acute fatal disease. It also causes congenital disease in humans and animals leading to abortion.

1

*Toxoplasma* antibodies were detected in Indian camels by Gill and Prakash (1969), in Afghanistan by Kirmse and Mohanbabu


Manal (1995) demonstrated the presence of developmental stages of Toxoplasma gondii in camel meat and isolated Toxoplasma oocysts from kittens fed cameline meat.

The wide-spread prevalence of Toxoplasma antibodies among sudanese camels warants a closer look into its economic impact as well as its public health significance especially among nomads who consume raw cameline milk and liver.

The genus Sarcocystis was originally described as “Milky white threads” (Miescher’s tubules) in the skeletal musculature of a mouse by Miescher (1843).

The complete life cycle of Sarcocystis was discovered in 1972 by Fayer; Heydorn and Rommel. Sarcocystis has an obligatory heteroxenous life cycle with herbivores acting as intermediate hosts and carnivores as definitive hosts.

Sarcocystis is pathogenic for intermediate hosts, causing both acute and chronic disease known as Sarcocystosis; it causes significant economic losses to the livestock industry, it affects the
reproductive system of animals and it can also be an important cause of human illness.

*Sarcocystis* cysts of camels were first discovered in Egypt by Mason (1910). Hilali and Mohamed (1980) defined the domestic dog as a definitive host for *Sarcocystis cameli*. Manal (1995) reported dogs as definitive hosts for two species of *Sarcocystis* of the camel; *Sarcocystis cameli* and *Sarcocystis sp.*

Camel sarcocystosis is widespread in the world, 82.5% in Somalia (Borrow et al., 1989), 88.5% in Saudi Arabia (Fatani et al., 1996), 91.6% in Iraq (Latif et al., 1999) and 45.5% in Ethiopia (Woldemeskel et al., 2001). In the Sudan *Sarcocystis* was demonstrated microscopically by Ginawi and Shommein (1977) who found 4.5% prevalence in camels, whereas investigation by Hussein and Warrag (1985) revealed 81%. In Khartoum area the prevalence of *Sarcocystis* was 11% in stray dogs (Hussein and Warrag, 1983).

1.2. **Taxonomy:**

The classification followed in this work is that of the committee on Systematics and Evolution of the Society of Protozoologists (Levine et al., 1980).

**Phylum:** Apicomplexa Levine, 1970;
**Class:** Sporozoa Leuckart, 1879;
**Subclass:** Coccidia Leuckeart, 1879;
Order: Eucoccidiidia Leger and Duboseq, 1910;
Suborder: Eimeriina Leger, 1911;
Family: Sarcocystidae Poche, 1913;
Subfamily: Sarcocytinae Poche, 1913;
Genus: Sarcocystis Lankester, 1882.
Subfamily: Toxoplasmatinae Biocca, 1956;
Genus: Toxoplasma Nicolle and Manceaux, 1908.

1.3. Morphology:

1.3.1. Toxoplasma:

*Toxoplasma* oocyst is di-sporocystic, tetra-sporozoic, spherical to subspherical in shape 11-13 µm x 9-11 µm in diameter and without a micropyle. The oocyst wall is thin (1 µm), fragile and contains two colourless layers. The sporocyst measures 8-9 µm x 5-6.5 µm without stieda body, but the residium body is present (Frenkel *et. al.*, 1970; Sheffield and Melton, 1970).

*Toxoplasma* tissue cyst may measure up to 100 µm in diameter and contains up to 60,000 organisms. Bradyzoites in the cyst are closely packed together, lancet-shaped and each possesses a terminal nucleus. The cyst-wall has an irregular surface lined by a granular layer (Frenkel 1973).

1.3.2. Sarcocystis:

*Sarcocystis* sporocysts have an ellipsoidal shape, smooth wall with one side being more convex than the other. Each sporocyst
contains four elongated banana-shaped sporozoites and sporocystic residium (Fayer, 1972).

The *Sarcocystis* cysts may be thread-like, spindle or egg-shaped. In many *Sarcocystis spp.*, septa develop from the granular layer, extending into the interior of a sarcocyst and divide it into unites of variable size containing metrocytes and/or bradyzoites. Two distinct regions are recognizable within the cyst, the peripheral region containing metrocytes which by endodyogeny produces two daughter cells. These cells after several further replications give rise to banana–shaped bradyzoites (Scholtyseck, 1973).

The size of *Sarcocystis* sporocysts and *Sarcocystis* cysts varies according to species. The thickness and structure of the cyst-wall may vary within each species as well as within different stages of maturity (Levine, 1986). The sarcocyst has a primary cyst-wall which is folded into protrusions; it varies in length, shape and micromorphology according to the species.

1.4. **Life cycle:**

1.4.1. *Toxoplasma* :

*Toxoplasma gondii* may follow a life cycle that is either monoxenous or heteroxenous. Oocysts from faeces of felids are infectious to other felids as definitive hosts and numerous warm-blooded animals as intermediate hosts. In felids that ingest sporulated oocysts, asexual stages and their gamonts develop in the intestinal
epithelial cells. After fertilization of the macrogametes, the oocysts develop, then released into the intestinal lumen and passed into the environment with the faeces. Sporogony occurs outside the host and leads to the development of infectious oocysts.

In intermediate hosts, *Toxoplasma gondii* undergoes two phases of asexual development. In the first phase, tachyzoites can enter host cells via phagocytosis and or active invasion. Tachyzoites occur within vacuoles in their host cells with a definitive space between the parasite and vacuole wall. From the site of primary infection in the gut, tachyzoites spread from cell to cell and are disseminated by the lymphatics and blood stream. Multiplication of the tachyzoites within cells is by endodyogeny. Tissue-cyst formation is characterized by slowing multiplication of the tachyzoites leading to an accumulation of bradyzoites within the large intracellular cyst-like structures. Multiplication of bradyzoites is by endodyogeny.

Tissue-cysts have a high affinity for neural and muscular tissues. They are located predominantly in the central nervous system, the eye, skeletal and cardiac muscles (Dubey, 1998b). Also, they may be found in visceral organs, such as lungs, liver and kidneys (Dubey, 1980).

There are three infectious stages in the life cycle of *Toxoplasma gondii*; tachyzoites, bradyzoites contained in tissue-cysts, and
sporozoites contained in sporulated oocysts. All three stages are infectious for both definitive and intermediate hosts.

1.4.2. *Sarcocystis*:

*Sarcocystis* has an obligatory two-host life cycle with herbivores acting as intermediate hosts and carnivores as final hosts. The intermediate hosts acquire infection by ingesting sporocysts that are shed with the faeces of infected definitive hosts. Sporozoites excyst in the intestine of the intermediate host and enter the endothelial cells of medium sized arteries in various organs, whereby develop into first generation meronts. Tachyzoites from these meronts give rise to second generation meronts in the endothelial cells of capillaries throughout the body of the host. At this stage acute sarcocytosis occurs. Tachyzoites from these second generation meronts give rise to the cyst stage in the brain, striated and cardiac muscles. Immature cysts contain metrocytes, which through endodyogeny give rise to bradyzoites in mature cysts, (chronic stage), that infect the final host through carnivorism.

After ingestion by the final host, bradyzoites develop directly into gamonts in the *Lamina propria* of the small intestine without producing meronts (Fayer, 1974). Following conjugation of gametes, thin-walled oocysts are formed which then sporulate within the host.

The oocyst-wall often breaks releasing the sporocysts in the lumen of the intestine. In this way, fully sporulated sporocysts are usually shed.
with the faeces, and are readily infective for the intermediate herbivore hosts.

1.5. *Toxoplasma* and *Sarcocystis* species from camels:

*Toxoplasma gondii* is the only valid species of the genus *Toxoplasma*. The recent molecular epidemiological studies have provided evidence that there are two clonal lineages within *Toxoplasma gondii*, one comprising strains that are virulent in mice and another comprising strains that are avirulent in mice (Johnson, 1997).

Mason (1910) in his original report of the occurrence of *Sarcocystis* infection in Egyptian camels described two types of tissue-cyst. In 1979 Abdel Ghaffar *et. al.*, described *Sarcocystis* cyst that had smooth wall without striations.

Hilali and Mohamed (1980) described the sarcocyst from camels that had thick striated wall.

On the other hand, Manal (1995) detected two types of *Sarcocystis* tissue-cysts in Sudanese camels. One sarcocyst type measured 72.5 – 264 µm x 9.9 – 29.5 µm with thin smooth non-striated wall. The other sarcocyst type measured 73-155 µm x 23-29.5 µm and had a thick striated cyst-wall. Two species of *Sarcocystis* sporocysts were isolated from dogs fed on cameline meat; *Sarcocystis cameli* and *Sarcocystis sp.* (Manal 1995).
1.6. Transmission:

1.6.1. *Toxoplasma*:

*Toxoplasma gondii* has a broad range of potential routes of transmission; by oral ingestion of infectious oocysts from the environment, by oral ingestion of tissue-cysts contained in raw or undercooked meat or infected animal tissues, by ingestion of milk contaminated with tachyzoites (Rieman *et. al.*, 1975), by transplacental transmission of tachyzoites (Dubey & Beattie, 1988), and by blood transfusion or organ transplantation (Dubey, 1993).

1.6.2. *Sarcocystis*:

The intermediate hosts acquire the infection by ingesting sporocysts or sporulated oocysts that are shed in the faeces of infective definitive hosts.

Carnivorism is the main route for transmission of *Sarcocystis* to the definitive hosts.

1.7. Pathogenicity:

1.7.1. *Toxoplasma*:

*Toxoplasma gondii* usually parasitizes both definitive and intermediate hosts without producing clinical signs. The pathogenicity depends upon major factors; the virulence and number of the infecting strains, the immunological status and genetic composition of the animals.
Toxoplasmosis is one of the most common parasitic disease of man and animals. The pathogenicity of toxoplasmosis is always related to extraintestinal phase of development. Necrosis and focal acute or chronic inflammatory reactions may have occurred by actively replicating asexual stages (Frenkel, 1973).

Most *Toxoplasma* infections in man and animals are mild and consequently asymptomatic.

However, the most serious manifestation of the disease occurs in congenital foetal involvement in human being. Abortion is a common sequele in severe infections acquired early in pregnancy and if a child is born alive he may suffer from serious mental retardation within a few weeks after birth. Necrotizing retinochoroiditis is reported as the commonest clinical manifestation of congenital toxoplasmosis in human being.

*Toxoplasma gondii* is a major cause of abortion in sheep in Newzealand and England (Hartley and Marshall, 1957). It causes abortion in goats (Dubey, 1981 a&b). *Toxoplasma* infection is an important contributor to ovine infertility and perinatal mortality (Hartley and Boyes, 1964).
Sanger *et. al.*, (1953) recorded an outbreak of toxoplasmosis in a herd of Brown Swiss cattle. The herd showed dyspnoea, coughing, sneezing, nasal discharges, trembling and shaking of head.

In dromedary, a case report of natural infection of toxoplasmosis was first reported by Hagemoser *et. al.*, (1990). The camels showed dyspnoea, anorexia, abortion and death of foetus.

1.7.2. *Sarcocystis*:

Most species of *Sarcocystis* are not pathogenic to their final hosts, but they are pathogenic to their intermediate hosts. The pathogenicity was affected by the number of sporocysts ingested by intermediate hosts. Acute sarcocystosis is caused by schizogonous phases in vascular endothelium or other cells of various organs while chronic disease results from sarcocyst formation in the brain, striated or cardiac musculature of intermediate hosts.

Dalmeny disease is a naturally occurring chronic disease of cattle caused by *Sarcocystis* (Corner *et. al.*, 1963). This disease is characterized by emaciation, decrease in milk yield, dyspnoea, abortion, exophthalmia and submandibular oedema.

Abortion is a common sequel for *Sarcocystis* infection in cows experimentally infected with *Sarcocystis* (Fayer *et. al.*, 1977). Sarcocystosis in cows reduced milk production (Fayer *et. al.*, 1983).
Heavy experimental infection of calves with *Sarcocystis* has resulted in mortality after one month (Johnson *et. al.*, 1975). The clinical signs included were anorexia, pyrexia, anaemia and loss of weight.

Goat experimentally infected with *Sarcocystis* showed anaemia, weight loss, abortion and death (Dubey *et. al.*, 1981).

*Sarcocystis* can cause abortion, weight loss and death in sheep (Leek and Fayer, 1978).

Overall, sarcocystosis of intermediate hosts can be a highly significant disease; but clinical signs are rarely observed. The most significant effect is the presence of sarcocysts in muscles of food animals which results in downgrading or condemnation of carcasses.

1.8. Survival of sporocysts :

1.8.1. *Toxoplasma* :

Sporulated oocysts of *Toxoplasma gondii* are very resistant to environmental conditions. They survive short periods of cold and dehydration. They remain infectious in moist soil or sand for up to 18 months (Frenkel, 2000). Under laboratory conditions, sporulated oocysts survived storage at 4°C for up to 54 months and freezing at –10°C for 106 days; however, they were killed within 1-2 minutes by heating to 55-60°C (Dubey, 1998c). Sporulated oocysts are highly impermeable and, therefore, are also very resistant to disinfectants (Frenkel, 2000; Kuticic and Wikerhauser, 1996).
1.8.2. *Sarcocystis:*

*Sarcocystis cruzi* sporocysts are able to survive in most environmental conditions for several months and that the fluctuations of the daily ambient temperature is likely to influence the viability of the sporocysts (Savini et. al., 1996).

Heat treatment is the most effective means of killing *Sarcocystis neurona* sporocysts; sporocysts heated to 55°C for 15 minutes or 60°C for one minute were rendered non-infective to mice. While treatment with disinfectants (100% bleach, 2% chlorhexidine, 1% betadine, 12.5% phenol, 10% formaline or 2.9% ammonia) were not effective in killing sporocysts (Dubey et. al., 2002).

1.9. **Survival of tissue-cysts:**

1.9.1. *Toxoplasma:*

Although *Toxoplasma* tissue-cysts are less resistant to environmental conditions, yet they are relatively resistant to changes in temperature and remain infectious in refrigerated (1-4°C) carcasses or minced meat for up to 3 weeks (Dubey et. al., 1990). Tissue-cysts also survive freezing at temperature between –1°C and –8°C for longer than a week, but most tissue-cysts are killed at temperature of –12°C or lower (Kotula et. al., 1991). Tissue- cysts in meat are killed by heating to 67°C (Dubey, 2000), but remained viable at 60°C for 4
minutes and at 50°C for 10 minutes depending upon duration of cooking (Dubey et. al., 1990).

Toxoplasma tissue-cysts were killed in 6% NaCl solution at temperatures from 4 to 20°C, but survived in aqueous solutions with lower concentrations of salt for several weeks (Dubey, 1997).

1.9.2. Sarcocystis:

Sarcocysts of Sarcocystis levinei are rendered non-infective by heating to 65°C or by freezing at –4°C for 48 hours (Srivastava et. al., 1986).

Freezing to –18°C and –24°C and cooking were effective in activating Sarcocystis sp. in guanaco (Gorman et al. 1984).

Pork meat infected with Sarcocystis miescheriana requires cooking at minimum 70°C for 15 minutes or freezing at –4°C for 2 days or –20°C for one day for making it safe for consumption (Saleque et. al., 1990).

1.10. Diagnosis:

1.10.1. Toxoplasma:

Diagnosis of toxoplasmosis is seldom based on identification of the causal organism isolated from infected tissues because of the difficulty and time consumed in repeated mouse passage. In the main, diagnosis is based on a correlation of clinical and serological findings.
The most useful and widely studied methods for serodiagnosis are: dye test (Sabin and Feldman, 1948), indirect immunofluorescence antibody test (Remington et. al., 1968), direct and indirect haemagglutination test (Jacobs and Lunde, 1957). Diagnosis of Toxoplasma is usually done by ELISA, using a culture which may take up to 3 weeks. MRT scan or brain biopsies are also used to identify cysts in the brain. Recently, polymerase chain reaction (PCR) has been used for early diagnosis of toxoplasmosis, it is highly sensitive and specific (Shibata et. al., 1995).

1.10.2. Sarcocystis:

Diagnosis of acute sarcocystosis presents many problems because the clinical signs (anorexia, weight loss, fever and anaemia) are non-specific. Infections are diagnosed by direct demonstration of organisms (meronts, cysts or cystozoites) in host-tissues collected at necopsy. The Sarcocystis cysts contain metrocytes and show typical septa inside the cyst which are not present in the other tissue-cysts except those of Frenkelia. Sarcocysts can be detected in the musculature of intermediate hosts by gross examination, unstained squash preparations, digestion methods and histological studies. Tissue digestion methods are sensitive although speciation of bradyzoites is not possible. Ultrastructural examination is precise although tedious, time-consuming and expensive.
Several immunological tests have been successfully used to detect anti-*Sarcocystis* antibodies in experimentally infected animals; including the indirect fluorescent antibody test (Arru et. al., 1978) and the indirect haemagglutination test (Lunde and Fayer, 1977). O’Donoghue and Weyreter, (1983) used the enzyme-linked immunosorbent assay to detect circulating antigens in experimentally infected pigs and mice.

In the final hosts, diagnosis of *Sarcocystis* is based mainly on the detection of sporocysts in faeces

**1.11. Objectives of the study:**

The present study was initiated to determine the following:

a. The sero-prevalence of *Toxoplasma* antibodies in Sudanese camels

b. Congenital toxoplasmosis on camels.

c. The presence of *Toxoplasma gondii* tachyzoites in milk of infected camels.

e. The effect of acute sarcocystosis on calf-camels.
CHAPTER II

General Materials
and
Methods
2. General Materials and Methods

2.1. Detection of *Toxoplasma* antibodies in Sudanese Camels:

Blood was obtained from camels owned by nomads in different parts in the Sudan (South Kordofan, El Gedarif, El Hamra, El Butana, El Shouak, River Nile Province). Blood samples were encountered by jugular venopuncture into a plain vacutainer (Becton – Dickinson, France). The blood samples were left to clot overnight at 4ºC and sera were decanted into plastic tubes. Separated sera were stored at – 20ºC until used. The age of the camels was estimated from the dentition.

A total of serum samples were tested for *Toxoplasma* antibodies by the latex agglutination test (LAT) using Toxolatex test kit (Spinreact comp. Spain). The test was performed on black slide.

The toxo-latex reagent is a suspension of polystyrene latex particles coated with soluble *Toxoplasma gondii* antigen. Latex particles allow visual observation of the antigen – antibody reaction.

Each serum sample was screened at two fold dilution ranging from 1 : 8 to 1 : 2048.

The non-parametric statistical test Chi-square was used to compare between the sero-prevalence of antibodies in the different
locations, age-groups and sex-groups. It was used in accordance with the nature of the data which follows a binomial distribution and takes the form of proportion.

2.2. Experimental animals:

2.2.1. Dogs and cats:

Five weaned puppies (4-week-old) and five weaned kittens (6-week-old) were used in this study. They were reared in separate Kennels, at the Department of Pathology and Diagnosis, Central Veterinary Research Laboratories.

The puppies & kittens were proven to be free of coccidian oocyst and / or sporocyst by daily faecal examination for two consecutive weeks prior to the experimental infection. During the experimental period the puppies and the kittens were kept on preboiled milk mixed with bread.

2.2.2. Camels:

Six pregnant she-camels, four she-camels with their suckling calves and four naïve calf-camels (4-6-week-old) were obtained from camel’s herderer at the outskirts of Omdurman.

All camels were serologically negative for *Toxoplasma gandii*, as determined by latex agglutination test using toxolatex test kit (Spinreact Comp., Spain).
The camels were kept in animal pens at the Central Veterinary Research Laboratories. During the experimental period, the camels were fed alfalfa hay and concentrates.

The camels were proved free from endoparasites by standard faecal floatation and sedimentation techniques. Whole blood was collected from each camel at weekly intervals, throughout the experimental period.

Blood samples were collected into heparinized vacutainer tubes (Becton-Dockinson, France) for haematological studies. The experimental camels were divided into three groups;

**Group I**: This group contained six pregnant she-camels, it was divided into three subgroups; A, B and C. Each subgroup contained two she-camels, one camel at the first half of pregnancy and the other one at the second half of pregnancy

**Group II**: This group contained four she-camels with their suckling calves.

**Group III**: Contained four naïve calf camels.

2.3. **Preparation of meat samples**:

Meat samples (composite of oesophagus, tongue, heart, lymph nodes, diaphragm and skeletal muscles) were collected from 13 camels slaughtered at El-Gedarif slaughter-house, mid-Eastern Sudan.
The meat was cooled, transported to Khartoum in an ice-pot. The samples were minced and fed to the experimental animals (dogs and cats) on the same day of slaughtering of camels.

**2.4. Experimental infection of dogs and cats:**

The camel meat samples were minced, then fed to the puppies and kittens. Each puppy was fed with 400gm of minced meat and each kitten was fed with 100 gm of minced meat.

One puppy and one kitten were not fed with the camel meat and were considered as experimental controls.

**2.5. Detection of oocysts and/or sporocysts in faeces of dogs and cats:**

Faecal samples were collected daily from each dog and cat. Faecal samples were examined for the presence of oocysts and/or sporocysts by sugar floatation technique(Dubey *et. al.*, 1970). The faeces were suspended in 10 times its volume of water and then centrifuged at 1.000G for 10 minutes. The supernatant liquid was discarded and the sediments were resuspended in sucrose solution of 1.15 specific gravity (with 0.8% phenol as a preservative). It was then centrifuged at 1.000G for 10 minutes.

One drop of the supernatant was aspirated from the surface, placed on a slide and covered with a glass slip. The drop was examined by light microscope.
When the dogs and cats passed out oocysts and/or sporocysts in their faeces, 1 ml of supernatant was aspirated from the surface, placed in a tube and washed twice by adding distilled water and centrifuged at 4000G for one minute. The resultant sediments were put into bottles containing 2.5% potassium dichromate. The bottles were labelled and kept in a refrigerator at 4°C.

2.6. Sporulation of oocysts voided by experimental animals:

Freshly passed faeces containing unsporulated oocysts were collected and placed in shallow glass petri dishes containing aqueous solution of 2.5% potassium dichromate at 20-30°C. Each sample was aerated once a day for 5 hours using an aquarium aeration pump.

2.7. Isolation of Toxoplasma oocysts:

Oocysts shed by kittens were collected and left to sporulate as described above (2.6). A pool of sporulated oocysts in minimal volume of saline solution (0.9% NaCl) were mixed with a combination of penicillin (1,000/U) and streptomycin (100 mg)/ml of saline solution. The samples were left for one hour at room temperature and then were inoculated subcutaneously into each of five albino mice.

After eight weeks these mice were killed and their brains were ground and mixed with a combination of penicillin and streptomycin
and then inoculated s/c into ten albino mice. After eight weeks the mice were killed and their brains were fed to ten naive kittens (6-week-old).

Faecal samples were collected daily from these kittens for the detection of *Toxoplasma* oocysts using sugar floatation technique.

2. 8. **Preparation of *Sarcocytis* inoculum:**

The puppies, fed cameline meat, were killed after twenty nine days and their intestinal mucosae were scraped into a beaker. The sporocysts were recovered using the method described by Box and Smith (1982).

The mucosal scrapings were diluted with 10 times their volume of artificial digestive fluid (Pepsin 0.65%, w/v; NaCl 0.86%, w/v; HCl 1%, v/v; in water) and stirred up for one hour at 37°C. The mixture was then centrifuged and washed twice in water.

The resulting sediment was resuspended with 10 times its volume of 0.4% w/v trypsin (1 : 300) in Ringer’s solution, pH 7.4. After a further period of digestion for one hour, at 37°C, the material was washed by centrifugation twice in Ringer’s solution. The final sediment was stored in several times its volume of 2.5% potassium dichromate at 4°C until used for the experimental infection of calf-camels.
2.9. Experimental infection of camels with *Toxoplasma gondii* oocysts:

Two pregnant she-camels (subgroup A) were inoculated orally with $1 \times 10^5$ sporulated *Toxoplasma* oocysts. One million sporulated *Toxoplasma* oocysts were inoculated orally into another two pregnant she-camels (subgroup B). Subgroup C served as noninoculated control.

Three she-camels, from group II, were inoculated orally with $5 \times 10^5$ sporulated *Toxoplasma* oocysts each and one she-camel from this group was kept as an experimental control.

2.10. Experimental infection of calf-camels with *Sarcocystis* sporocysts:

Three weaned calf-camels, group III, were infected orally with *Sarcocystis* sporocysts which were prepared as described above (2.8). The sporocysts were washed from potassium dichromate in which they were preserved. The sporocysts counting was performed using Mcmaster chamber.

Each camel received $1 \times 10^2$, $1 \times 10^5$ or $1 \times 10^6$ *Sarcocystis* sporocysts through a stomach tube. One calf-camel was not dosed with *Sarcocystis* sporocysts and was left as an experimental control.

2.11. Detection of *Toxoplasma* antibodies:

Sera samples were collected from each camel at weekly intervals, throughout the experimental period for detection of
Toxoplasma antibodies by agglutination test on slide using Toxo-latex Kit (Spinreact Comp., Spain).

The Toxo-latex reagent is a suspension of polystyrene latex particles coated with soluble Toxoplasma gondii antigen. Latex particles allow a visual observation of the antigen – antibody reaction. Each serum sample was screened at dilutions ranging from 1 : 8 to 2048.

2.12. Histological methods :

Tissue samples (lung, heart, diaphragm, tongue, oesophagus, spleen, kidney, liver, rumen, reticulum, omasum, abomasum, small and large intestine, mesentric and gastric lymph nodes, brain, spinal cord, eyes, aorta, coronary arteries and skeletal muscles) from the suckling calf-camels and from the infected new-borne calf-camels, were fixed in neutral buffered 10% formalin. Paraffin embeded tissues were cut at 5 µm, stained with haematoxylin and eosin (H & E) and examined by light microscope for detection of histological changes (Dubey et. al., 1981).

2.13. Examination of products of conception for Toxoplasma :

As the method described by Dubey, (1981b), portions of placenta, brain, heart, thigh muscles, spleen, lung, liver, diaphragm, eye, blood, mesentric lymph nodes, kidneys and stomach contents of
new-born calf-camels were homogenized separately in 5 volumes of 0.9% NaCl solution then 3 ml of the tissue suspension was mixed with 3 ml of antibiotic solution (penicillin/streptomycin). One ml of the mixture was inoculated IP into three albino mice.

2.14. Examination of mice for Toxoplasma infection:

Impression smears of brain, mesentric lymph nodes, lungs and livers of mice were examined for *Toxoplasma gondii* 6-8 weeks after inoculation inorder to detect *Toxoplasma* cysts. The brains of mice were fixed in 10% formal saline for histological examination. Latex agglutination test was used to detect antibodies to *Toxoplasma* in sera of mice.

2.15. Examination of calf-camels tissues for encysted *Toxoplasma gondii*:

50 g each of liver, kidneys, thigh muscles, brain, heart and diaphragm were homogenized in saline solution and then digested in acid pepsin solution for 90 minutes at 37°C. After washing in saline solution a homogenate of each organ was inoculated IP into three mice as described by Dubey, (1980).

2.16. Haematological methods:

Whole blood was collected from each camel at weekly intervals, throughout the experimental period. Jugular venopuncture blood samples were collected into heparinized vacutainer tubes (Becten-Dikinson, France). Haematological studies were conducted as described by Jain (1986).
2.16.1. Erythrocyte and leukocyte counts:

A rubber tube fitted with a plastic mouthpiece was attached to the upper end of the pipette. By gentle suction, blood was drawn into the stem of pipette up to the mark 0.5. The tip was then wiped for cleaning and the diluting fluid was drawn into the pipette to the 11 or 101 mark, depending on the type of the pipette used. Hayem’s solution is composed of 5 gm. Sodium sulphate, 1 gm sodium chloride, 0.5 gm mercuric chloride and 200 ml distilled water. Turek’s solution was used for dilution of blood for leukocyte count. The solution is composed of 1 ml acetic acid, 99 ml distilled water and a few drops of gentain violet. The fluid in the pipette was mixed thoroughly by rotation. The edge of the platform of the counting chamber was touched with the tip of the pipette to allow the fluid to flow under the coverslip by capillary action. The lower-power objective of the microscope used for the white-cell count in the four corners of primary squares. The count was multiplied by a factor of 50 to obtain the number of cells in 1 ml of blood. The high dry objective was employed in making the erythrocyte count. The number of cells in five secondary squares was determined and multiplied by a factor of 10,000. The results were converted into S1 units.

2.16.2. Estimation of haemoglobin concentration:

One tenth normal HCl was introduced into the central tube of the Sahli’s haemoglobinometer up to the mark 10. The blood sample
was sucked up to the mark 0.02 ml in the special pipette and was blown gently into the acid, mixed and allowed to stand for 3 minutes. Distilled water was added drop wise until the tint in the dilution tube was similar to that of the standard. The black graduation was red. It indicated the percentage of haemoglobin in the examined blood. The values were converted into g/dl.

2.16.3. Determination of packed cell volume (PCV):

Two haematocrit tubes were filled with blood sample. They were sealed with crisytaseal. The blood sample was centrifuged for 15 minutes at 2500 G. using a microcentrifuge (Hawkeshy and Sons, UK). The volume of the cells was read as a percentage of the total volume of the blood using the supplied ruler.

2.16.4. Differential count of white blood cells:

Blood smears on slides were fixed with methyl alcohol, then stained with Giemsa stain diluted 1 : 10 for 30 minutes. The differential leukocyte count was conducted under oil immersion objective and done on 100 cells on the bottle field method. The results were reported for each leukocyte type as percentage of total count.

2.17. Examination of milk for *Toxoplasma gondii*:

Milk of infected she-camels was examined for *Toxoplasma gondii* two weeks after infection. One ml of milk obtained from each she-camels was inoculated IP into each of three mice as described by Dubey (1980).
CHAPTER III

Sero-prevalence of *Toxoplasma gondii* antibodies in Sudanes camels.
3. Sero-prevalence of *Toxoplasma gondii* antibodies in Sudanes Camels

3.1 Introduction:

*Toxoplasma gondii* is widely distributed as intracellular parasite. It has very low host specificity, and thus it is capable to infect many mammalian and avian host species. Felines act as the definite hosts in which full sexual reproductive phase is completed.

*Toxoplasma gondii* is prevalent in most areas of the world. In camels, *Toxoplasma* antibodies were reported in India (Gill, Brakash, 1969), in Afghanistan (Kirmse, Mohanbabu, 1986), in Saudi Arabia (Afzal, Sakkir, 1994) in Egypt (Abu-zeid, 2002) and in the Sudan (Abbas *et al.*, 1987; Bronstein and Musa, 1987; Elamin *et al.*, 1992).

The aim of this study was to determine the prevalence of *Toxoplasma gondii* antibodies among camels of the Sudan.

3.2. Materials and Methods:

Blood samples were obtained from camels owned by nomads in different area in the Sudan. Sera were collected from camels as described in Chapter II, section 2.1.

Separated sera were decanted into plastic tubes and then stored at –20°C until used.
The age of camels was estimated from the dentition.

A total of 588 serum samples were tested for *Toxoplasma* antibodies by the latex agglutination test using Toxolatex test kit. Each serum sample was screened at two fold dilution ranging from 1 : 8 to 1 : 2048. The sera were collected from different areas; 256 serum samples from El Gedarif, 175 serum samples from Kordofan, 49 serum samples from River Nile, 49 serum samples from El Shawak, 43 serum samples from Butana and 16 serum samples from El-Hamra. The data were analyzed statistically by the non-parametric statistical Chi-square. This test was used to compare between the seroprevalence of antibodies in different locations, age groups, sex groups and antibody titers. It was used in accordance with the nature of the data which follows a binomial distribution and takes the form of proportion.

3.3. Results:

Out of 588 camels surveyed for *Toxoplasma* antibodies, 363 camels were seroreactive (61.7%).

Table 1 and Figure 1 shows the frequency distribution of *Toxoplasma gondii* antibodies among six surveyed locations. Results shows that the prevalence as follows: Butana 51%, N.Kordofan 60%, Gedarif 68%, Elshawak 57%, River Nile 51% and El Hamara 43%.

Table 1 also shows a seroreactivity correlated with significance between the surveyed locations (P<0.05). However, when Butana, El
Figure 1:

The distribution of sero-positivity in the serum samples

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of positive</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>butana</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Kordofan north</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>ElGedarf</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>ELShwak</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>river nile</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Elhamra</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>30%</td>
</tr>
</tbody>
</table>
Table 1:

Sero-prevalence of *Toxoplasma* in the different locations from which the samples were collected

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample</th>
<th>Positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butana</td>
<td>43</td>
<td>22</td>
<td>51</td>
</tr>
<tr>
<td>North Kordofan</td>
<td>175</td>
<td>106</td>
<td>60</td>
</tr>
<tr>
<td>Gedarif</td>
<td>256</td>
<td>175</td>
<td>68</td>
</tr>
<tr>
<td>ElShawak</td>
<td>49</td>
<td>28</td>
<td>57</td>
</tr>
<tr>
<td>River Nile</td>
<td>49</td>
<td>25</td>
<td>51</td>
</tr>
<tr>
<td>ElHamara</td>
<td>16</td>
<td>7</td>
<td>43</td>
</tr>
</tbody>
</table>

Pearson chi2(5) = 11.8998   Pr = 0.036
Gedarif, El Shawak were considered as one study area and North Kordofan and El Hamra were taken as one area, no significant difference in seroprevalence was detected between the three locations (P>0.05) Table 2.

Figure 2 shows seroprevalence of antibodies against *Toxoplasma* in the three locations.

Table 3 shows a highly significant difference between the seroprevalence in different age groups in the Total samples (P<0.01).

Table 4 summarized the titers frequency for the positive animals in different age groups, which is also shown in figure 3. Camels under one year of age have a high titer at 1 : 1024, the other age group showed titers ranged between 1 : 512 and 1 : 128.

No significance correlation was established between the seroprevalence in male and female in Butana (Table 5), North Kordofan (Table 6), River Nile (Table 7) and the total samples (Table 8). P > 0.05.

**3.4. Discussion:**

In this study *Toxoplasma gondii* antibodies were detected in six locations of camels in the Sudan using latex agglutination test.
Table 2:

Sero-prevalence of *Toxoplasma* in the Butana, North Kordofan and River Nile areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample</th>
<th>Positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butana</td>
<td>348</td>
<td>225</td>
<td>64</td>
</tr>
<tr>
<td>Kordofan</td>
<td>191</td>
<td>113</td>
<td>59</td>
</tr>
<tr>
<td>River Nile</td>
<td>49</td>
<td>25</td>
<td>51</td>
</tr>
</tbody>
</table>

Pearson chi\(^2\)(2) = 4.1727  Pr = 0.124
Figure 2:

The distribution of the sero-positivity in the sample after the sample area into large units.
Table 3:

Sero-prevalence of *Toxoplasma* in the different age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Sample</th>
<th>Positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 Year</td>
<td>206</td>
<td>111</td>
<td>53</td>
</tr>
<tr>
<td>1-3 Years</td>
<td>140</td>
<td>84</td>
<td>60</td>
</tr>
<tr>
<td>3-6 Years</td>
<td>61</td>
<td>46</td>
<td>75</td>
</tr>
<tr>
<td>7-9</td>
<td>101</td>
<td>67</td>
<td>66</td>
</tr>
<tr>
<td>10-12</td>
<td>64</td>
<td>45</td>
<td>70</td>
</tr>
<tr>
<td>&gt; 12</td>
<td>6</td>
<td>2</td>
<td>33</td>
</tr>
</tbody>
</table>

Pearson chi2(5) = 15.2586 Pr = 0.009
Table 4:

The titers frequency for the positive animals in different age groups

<table>
<thead>
<tr>
<th>Titer</th>
<th>&lt; 1 Year</th>
<th>1-3 Years</th>
<th>3-6 Years</th>
<th>7-9 Years</th>
<th>10-12 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0</td>
<td>2.380952</td>
<td>4.347826</td>
<td>2.985075</td>
<td>6.666667</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>4.761905</td>
<td>4.347826</td>
<td>0</td>
<td>13.33333</td>
</tr>
<tr>
<td>64</td>
<td>15.31532</td>
<td>3.571429</td>
<td>15.21739</td>
<td>16.41791</td>
<td>8.888889</td>
</tr>
<tr>
<td>128</td>
<td>27.92793</td>
<td>27.38095</td>
<td>19.56522</td>
<td>25.37313</td>
<td>26.66667</td>
</tr>
<tr>
<td>256</td>
<td>1.801802</td>
<td>3.571429</td>
<td>13.04348</td>
<td>13.43284</td>
<td>22.22222</td>
</tr>
<tr>
<td>512</td>
<td>23.42342</td>
<td>38.09524</td>
<td>21.73913</td>
<td>17.91045</td>
<td>6.666667</td>
</tr>
<tr>
<td>1024</td>
<td>31.53153</td>
<td>19.04762</td>
<td>17.3913</td>
<td>20.89552</td>
<td>6.666667</td>
</tr>
<tr>
<td>2048</td>
<td>0</td>
<td>1.190476</td>
<td>4.347826</td>
<td>2.985075</td>
<td>8.888889</td>
</tr>
</tbody>
</table>
Figure 3:

Frequency of the titer in the different age groups

Age groups

Percentage

< 1 Year 1-3 Years 3-6 Years 7-9 Years 10-12 Years

16 32 64 128 256 512 1024 2048
Table 5:

Sero-prevalence of *Toxoplasma* in the different sex groups in Butana area

<table>
<thead>
<tr>
<th>Sex</th>
<th>Samples</th>
<th>Positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>239</td>
<td>157</td>
<td>65</td>
</tr>
<tr>
<td>Male</td>
<td>107</td>
<td>68</td>
<td>63</td>
</tr>
<tr>
<td>Total</td>
<td>346</td>
<td>225</td>
<td>65</td>
</tr>
</tbody>
</table>

Pearson chi2(1) = 0.1487  Pr = 0.700
Table 6:

Sero-prevalence of *Toxoplasma* in the different sex groups in North Kordofan area

<table>
<thead>
<tr>
<th>Sex</th>
<th>Samples</th>
<th>Positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>80</td>
<td>46</td>
<td>57</td>
</tr>
<tr>
<td>Male</td>
<td>109</td>
<td>65</td>
<td>59</td>
</tr>
<tr>
<td>Total</td>
<td>189</td>
<td>111</td>
<td>58</td>
</tr>
</tbody>
</table>

Pearson chi2(1) =  0.0866  Pr = 0.769
Table 7:

Sero-prevalence of *Toxoplasma* in the different sex groups in River Nile area

<table>
<thead>
<tr>
<th>Sex</th>
<th>Samples</th>
<th>Positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>27</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>25</td>
<td>51</td>
</tr>
</tbody>
</table>

Pearson chi²(1) = 0.4949  Pr = 0.482
Table 8:

Sero-prevalence of *Toxoplasma* in the different sex groups

<table>
<thead>
<tr>
<th>Sex</th>
<th>Samples</th>
<th>Positive</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>346</td>
<td>218</td>
<td>63</td>
</tr>
<tr>
<td>Male</td>
<td>238</td>
<td>143</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>584</td>
<td>361</td>
<td>61</td>
</tr>
</tbody>
</table>

Pearson chi²(1) = 0.5100  Pr = 0.475
When Chi-square was used to compare between the seroprevalence in the six locations the difference was found to be significant (P<0.05). However when Butana, El Gedarif and El Shawak were considered as one study area (64.6%) and North Kordofan and El Hamra were taken as one area (59%), and River Nile as one area (51%), no significant difference in seroprevalence was detected between the three areas (P<0.05).

The results obtained are found to be similar to the findings of El amin et. al. (1992) who reported a prevalence rate of 67% in El Butana, this study reported a prevalence of 64.6% in El Butana, but 68% seroprevalent in El Gedarif.

Camels under one year of age have a high titer at 1 : 1024, the other age group showed titers ranged between 1 : 512 and 1 : 128. This result revealed the presence of a recent acute infection among young age groups more than old ones.

A highly significant difference between the sero-prevalence in the different age groups in the total sample was established (P<0.01). This result revealed a high prevalence among old animals. It is conceivable that the longer an animal lives, the greater the chance of it being exposed to Toxoplasma oocysts.

There were no significant sex-linked differences in the different sex groups.
CHAPTER IV

Congenital Toxoplasmosis
of Camels
4. Congenital Toxoplasmosis of Camels

4.1. Introduction:

Congenital toxoplasmosis is a result of vertical transmission of the parasite during pregnancy. The first record of congenital toxoplasmosis was made by Janku (1923) who described tissue-cysts of *Toxoplasma gondii* in the retina of an infant with congenital hydrocephalus and microphthalmia.

Congenital toxoplasmosis occurs only if the mother becomes infected during pregnancy. The temporary parasitemia in a primarily infected pregnant animal may result in invasion of the placenta by tachyzoites which then multiply within cells of the placenta. Eventually, some of these may cross the placenta and enter the foetal circulation or foetal tissues (Ebbesen, 2000).

The risk of intrauterine infection of the foetus and the severity of the disease depend on the time of maternal infection during pregnancy. The immunological competence of the mother during parasitaemia, the number and virulence of the parasites transmitted to the foetus and the age of the foetus at the time of transmission. Congenital infection is most severe if it is acquired in the first half of pregnancy; however, if transmission occurs at a late stage of pregnancy the effects on the foetus are less severe. (Dubey, 1981b).
Congenital toxoplasmosis may cause abortion, neonatal death or foetal abnormality (Remington, et. al., 1995). *Toxoplasma* infection is a major cause of sheep abortion and prenatal mortality (Hartley and Marshal, 1957). It causes abortion in goats (Dubey, 1981a&b). Hagemoser et. al., (1990) reported that congenital toxoplasmosis causes abortion in camels.

The aim of this study is to determine the effects of *Toxoplasma gondii* infection on pregnant she-camels with different infectious doses at different stages of pregnancy.

### 4.2. Materials and Methods :

*Toxoplasma gondii* oocysts were isolated from kittens that were fed cameline meat (composite of oesophagus, tongue, heart, lymph nodes, diaphragm and skeletal muscles) collected from 13 camels slaughtered at El-Gedarif slaughter-house.

The camel meat samples were minced, then fed to five weand kittens. Each kitten was fed with 100 gm of minced meat. one kitten was not fed with the camel meat and was considered as experimental control. Freshly passed faeces containing unsporulated oocysts were collected and placed in shallow glass petri dishes containing aqueous solution of 2.5% potassium dichromate at 20-30°C. Each sample was aerated once a day for 5 hours using an aquarium aeration pump.
A pool of sporulated oocysts in minimal volume of saline solution (0.9% NaCl) were mixed with a combination of penicillin (1,000/U) and streptomycin (100 mg)/ml of saline solution. The samples were left for one hour at room temperature and then were inoculated subcutaneously into each of five albino mice. After eight weeks these mice were killed and their brains were ground and mixed with a combination of penicillin and streptomycin and then inoculated s/c into ten albino mice. After eight weeks the mice were killed and their brains were fed to ten naive kittens (6-week-old).

Six pregnant she-camels were serologically negative for *Toxoplasma gondii*, as determined by the latex agglutination test, the camels were divided into three subgroups. Each subgroup contained two she-camels one was at the first half of pregnancy, while the other one was at the second half of pregnancy. Subgroup (A) contained two she-camels which were inoculated orally with 1x10^5 sporulated *Toxoplasma gondii* oocyst. Subgroup (B) contained two she-camels which received 1 x 10^6 sporulated *Toxoplasma gondii* oocysts. Subgroup (C) served as noninoculated control.

The experimental animals were observed periodically for clinical signs.

Blood samples were taken each week for haematology and serology examination for *Toxoplasma gondii*.
Foetal membranes were collected promptly after delivery. Portions of placenta and tissues from calf-camels (brain, liver, heart, tongue, oesophagus, lung, spleen, eye, thigh muscle, mesenteric lymph nodes, diaphragm, and kidney) were fixed in buffered 10% formaline for detection of histologic changes.

Portions of placenta and various tissues (brain, heart, lung, liver, diaphragm, spleen, eye, kidneys, mesenteric lymph nodes, stomach contents, blood and thigh muscles) from calf-camels were homogenized separately in 0.9% NaCl solution. The tissue suspension was mixed with penicillin/streptomycin solution. One ml of the mixture from each sample was inoculated IP into three albino mice.

Examination of mice for *Toxoplasma gondii* infection was made by impression smears of brain, mesenteric lymph nodes, lungs and livers of mice were examined for *Toxoplasma gondii* 6-8 weeks after inoculation in order to detect *Toxoplasma* cysts. The brains of mice were fixed in 10% formal saline for histological examination. Latex agglutination test was used to detect antibodies to *Toxoplasma* in sera of mice.

Tissues of the calf-camels (liver, kidneys, thigh muscle, diaphragm and brain) were examined for encysted *Toxoplasma gondii*, using the pepsin-acid digestion technique.
4.3. Results

All she-camels were clinically normal during the experimental period, except one which received $1 \times 10^6$ infective *Toxoplasma* oocysts at the first half of pregnancy. It showed nervous signs 10 days after infection, ran after other camels and bit them. These signs remained for 7 days then the camel became clinically normal. Macroscopic and microscopic lesions were found in all four calf-camels.

Details are as follows:

**Calf – camel I:**

The mother was infected with $1 \times 10^5$ *Toxoplasma* oocysts at the first half of pregnancy. The calf was born very weak with closed eyes and diarrhoea (Fig. 4). It needed assistance in suckling during the first 5 days. Then after it refused suckling and became weak, showed corneal opacity and died at day 12 of age.

**Macroscopic findings:**

Petechial haemorrhages were shown in brain and heart. Lungs showed emphyzema and haemorrhages. Kidneys were fragile and showed haemorrhage. Spleen and lymph nodes were enlarged and haemorrhagic.
Figure (4) : A photograph of calf-camel 1 at day of birth (Weak with closed eyes and unable to stand)
Microscopic findings:

Encephalitis with necrosis, mononuclear cells infiltration, congestion and *Toxoplasma* cyst were found in the brain. (Fig. 5). Liver showed necrosis, mononuclear cells infiltration heamorrhage and congestion (Fig. 6). Lung showed emphyzema, congestion and infiltration of mononuclear cells (Fig. 7). Haemorrhages and infiltration of lymphoid cells occurred in the kidneys (Fig. 8).

Calf-Camel 2:

The mother was infected with $1 \times 10^5$ infective *Toxoplasma* oocysts at the second half of pregnancy. The calf-camel appeared normal at birth but later showed diarrhoea. It was unable to stand and needed assistance in suckling (Fig. 9). It died at day 40 of age.

Macroscopic findings:

Haemorrhages were seen in the retina of the eye brain and kidneys. Tongue showed ulceration; lung showed emphyzema and haemorrhages. Necrotic foci and casiation existed in mesentaic lymph nodes and in renal lymph nodes respectively. Bloody oedema was found in the ventral abdominal wall and thrombus in the abdominal vein.

Microscopic findings:

*Toxoplasma* cyst, encephalitis with necrosis and mononuclear cells infiltration was found in the brain (Fig. 10). Vacculation, heamorrhages, and infiltration of mononuclear cells were found in liver.
Figure (5): A histosection in the brain of calf-camel 1 (H & E) x 100. Notice *Toxoplasma* cyst, necrotic foci and congestion of blood vessel.

Figure (6): A histosection in the liver of calf-camel 1 (H & E) x 100. Notice necrosis of hepatocytes, haemorrhages, congestion and infiltration of mononuclear cells.
Figure (7): A histosection in the lung of calf-camel 1 (H & E)x100. Notice congestion of blood vessel and emphyzema.

Figure (8): A histosection in the kidney of calf-camel 1 (H & E)x100. Notice infiltration of mononuclear cells and haemorrhages.
Figure (9) : A photograph of calf-camel 2 at day of birth
(Fig. 11). Oedema, haemorrhages, emphysema and infiltration of mononuclear cells were seen in the lungs (Fig. 12).

**Calf-Camel 3 :**

The mother was infected with $1 \times 10^6$ infective *Toxoplasma* oocysts at the first half of pregnancy. A dead full-term calf-camel was born.

**Macroscopic findings :**

Haemorrhage covered the left side of the brain. The lung showed petechial haemorrhage and was filled with frothy fluid. Flappy heart with frothy fluid inside and discoloration of liver were observed. Lymph nodes and spleen were enlarged. Kidneys showed haemorrhages.

**Microscopic findings :**

The brain showed congestion of blood vessels infiltration of mononuclear cells and focal necrosis (Fig. 13). Infiltration of mononuclear cells, necrosis and haemorrhages were found in the liver, (Fig. 14). Lung showed oedema, haemorrhages and infiltration of mononuclear cells (Fig. 15). Haemorrhages, necrosis and infiltration of mononuclear cells were found in kidneys (Fig. 16).

**Calf-Camel 4 :**

The mother was infected with $1 \times 10^6$ infective *Toxoplasma* oocysts at the second half of pregnancy. The calf was born weak with
Figure (10): A histosection in the brain of calf-camel 2 (H & E)x400. Notice *Toxoplasma* cyst and necrosis.

Figure (11): A histosection in liver of calf-camel 2 (H & E)x100. Notice necrosis of hepatocytes.
Figure (12) : A histosection in lung of calf-camel 2 (H & E)x100. Notice oedema, emphyzema and infiltration of lymphoid cells.

Figure (13) : A histosection in brain of calf-camel 3 (H & E)x100. Notice congestion of blood vessels and necrotic foci.
Figure (14) : A histosection in liver of calf-camel 3 (H & E)x100. Notice necrosis of hepatocytes haemorrhage and infiltration of lymphoid cells.

Figure (15) : A histosection in lung of calf-camel 3 (H & E)x100. Notice oedema, haemorrhage and infiltration of lymphoid cells.
Figure (16): A histosection in kidney of calf-camel (H & E)x100. Notice haemorrhage and infiltration of lymphoid cells.
closed eyes, unable to stand and had diarrhoea (Fig. 17). At day 4 after birth it refused suckling. It was killed 6 days after birth.

**Macroscopic lesions:**

Haemorrhages were seen in the brain, skeletal muscles, lung, lymph nodes, kidneys, spleen, tongue and eyes (Fig. 18). The lung showed emphyzema and the liver showed discoloration. The kidneys were fragile.

**Microscopic lesions:**

Focal necrosis and congestion of blood vessels were found in the brain (Fig. 19). The liver showed infiltration of lymphoid cells, haemorrhages, with diffused necrosis and vacuolation (Fig. 20). The lungs showed haemorrhages, oedema and infiltration of mononuclear cells (Fig. 21).

*Toxoplasma gondii* tachyzoites haemorrhages, congestion and infiltration of mononuclear cells (mainly eosinophils) were detected in intestines of four Calf-camels (Fig. 22). Also severe damage of epithelial cells were found.

*Toxoplasma* tachyzoites were found in all infected calf-camels and all infected mice (Fig. 23). But not isolated from mice inoculated with pepsin digests.

*Toxoplasma gondii* cysts were found in all infected mice (Fig. 24). The cyst measured 12.0 - 16.6 μm x 11 - 15.5 μm and was
Figure (17) : A photograph of calf-camel 4
Figure (18) : A photograph of calf-camel 4. Notice haemorrhage in eye.
Figure (19): A histosection in brain of calf-camel 4 (H & E) x100. Notice congestion of blood vessels and necrosis foci.

Figure (20): A histosection in liver of calf-camel 4 (H & E) x100. Notice necrosis of hepatocytes, inflammatory cells and haemorrhage.
Figure (21) : A histosection in lung of calf-camel 4 (H & E)x100. Notice oedema, haemorrhage and inflammatory cells.

Figure (22) : A histosection in intestine of calf-camel (H & E)x100. Notice congestion of blood vessel, infiltration of lymphoid cells and damage of epithelial cells.
Figure (23): A photomicrograph of *Toxoplasma* tachyzoites (H & E)x100.
Figure (24): A photograph of *Toxoplasma gondii* cyst in mouse brain (H & E)x400.
surrounded with an irregular thin cyst wall. The bradyzoites in the cyst were closely packed.

Focal necrosis and hemorrhages were found in the placentae of all infected she-camels; the characteristic lesions consist of white flecks or multiple white yellowish area of discoloration up to one cm in diameter (Fig. 25). The placentae were enlarged and fragile. Microscopic findings revealed focal necrosis, calcification, infiltration of mononuclear cells and hemorrhages.

The control she-camels remained healthy, they completed their pregnancy periods normally, and delivered healthy calves. The placentae were normal in size and feature. *Toxoplasma gondii* was not isolated from their placentae or their calves.

In all inoculated she-camels, a latex test titre developed 1 to 3 weeks after inoculation.

Antibody titres of calf-camels at birth were generally higher than those of their mothers (Fig. 26).

The haematologic values of the infected she-camels remained within the normal level during the experimental period.

**4.4. Discussion**

In this study the effect of *Toxoplasma* infection on calf-camel 1 (survive for 12 days) was more severe than the effect of *Toxoplasma* infection on calf-camel 2 which died at day 40 of age. On the other
Figure (25): A photograph of placenta. Notice haemorrhages, necrosis and enlargement of the size.
Figure (26): Toxoplasma antibody titres of she-camels and calf-camels at day of birth.
hand calf-camel 3 was born dead (full-term) while calf-camel 4 was born alive but weak. These findings were similar to those of Dubey (1981b) who found that goats experimentally infected during the first months of pregnancy delivered dead lambs, whereas the damage on lambs was less severe when ewes were infected during the later stage of pregnancy.

This study showed that, the outcome of *Toxoplasma* infection varied according to the number of parasites inoculated into pregnant she-camels since a she-camel which received \(1 \times 10^6\) *Toxoplasma* oocysts at first half of pregnancy delivered a full term dead calf. Whereas she-camel which received \(1 \times 10^5\) *Toxoplasma* oocysts at the first half of pregnancy delivered alive calf and died at 12 day of age.

Foetal brains and eyes were the most affected organs in all experimentally infected calf-camels. Diarrhoea was the main clinical symptom that was found in all calf-camels infected with *Toxoplasma gondii* tachyzoites; congestions, haemorrhages and infiltration of lymphoid cells (mainly eosinophil) were detected microscopically in the intestinal villi. Eosinophilic reaction indicates parasitic infection.

*Toxoplasma* cyst was identified in the brain of calf-camel 2.

Isolation of *Toxoplasma* tachyzoites and cysts from infected mice confirmed the congenital infection of calf-camels.
Toxoplasma tachyzoites were not demonstrated in histologic sections of placentae but isolated from mice inoculated with those placentae, this may be due to the presence of low number of tachyzoites in placentae.

The high infective dose inoculated into she-camel 3 led to nervous manifestations.

The results of this study indicate a relationship between the time of exposure of pregnant she-camels to Toxoplasma gondii infection and the pathogenicity on their offspring.
CHAPTER V

Detection of *Toxoplasma gondii* tachyzoites excreted in milk of experimentally infected camels
5. Detection of *Toxoplasma gondii* tachyzoites excreted in milk of experimentally infected camels

5.1. Introduction

Tachyzoites of *Toxoplasma gondii* have been found in milk of several intermediate hosts including sheep, goats and cows (Dubey, Beattie, 1988; Johnson, 1997; Remington and Desmonts, 1990).

A recent study showed that tachyzoites of *Toxoplasma* may occasionally survive for two hours in acid Pepsin solutions (Dubey, 1998a). This may be of public health significance for nomads who consume cameline milk raw.

The aim of this study is to determine the presence of *Toxoplasma gondii* tachyzoites in milk of camels infected with *Toxoplasma*.

5.2. Materials and Methods

Four she-camels with their suckling calves were obtained from camels herderer at the outskirt of Omdurman.

All camels were serologically negative for *Toxoplasma gondii* as described in chapter II section 2.2.2.

The camels were kept in animal pens at the Central Veterinary Research Laboratories, they were fed alfa hay and concentrates during the experimental period.
Three she-camels were inoculated orally with $5 \times 10^5$ sporulated *Toxoplasma gondii* oocysts. One she-camel was not infected and left as control.

*Toxoplasma gondii* oocysts were isolated from kittens fed cameline meat as described in chapter II section 2.6. Detection of *Toxoplasma* antibodies was done as described in chapter II section 2.10. Examination of milk for *Toxoplasma gondii* was applied as described in chapter II section 2.16.

Mice were killed 30 DAI and examined for *Toxoplasma* infection as described in chapter II section 2.13. Mice were considered uninfected when antibodies to *Toxoplasma gondii* were absent and when *Toxoplasma gondii* parasites were not demonstrated.

Suckling calf-camels were killed 50 days after infection of the mothers. Tissue samples from the suckling calf-camels were prepared for histological examination as described in chapter II section 2.11.

### 5.3. Results

*Toxoplasma* tachyzoites or cysts were found in six mice that were inoculated with milk obtained from two infected she-camels. *Toxoplasma gondii* antibodies were detected in mice sera. On the other hand, three mice and control did not show *Toxoplasma* infection; no tachyzoites were detected microscopically nor antibodies were detected serologically.
Direct agglutination tests for suckling calf-camels sera revealed the presence of *Toxoplasma gondii* antibodies 7-9 day after infection of their mothers. The antibody titers were low (1/8 – 1/16). Suckling calf-camels remained clinically normal during the experimental period (50 days).

In postmortem examination all suckling calf-camels showed enlargement of mesentric, inguinal and subscapular lymph nodes. Peticheal hemorrhages were found in the brain and lungs. Microscopic examination revealed the presence of *Toxoplasma gondii* tachyzoites in lungs, kidneys and hearts of all infected suckling calf-camels. *Toxoplasma gondii* cyst was detected in the brain of suckling calf-camel (Fig. 27 ). Lungs showed necrosis and infiltration of lymphoid cells. Kidneys showed hemorrhages and infiltration of lymphoid cells.

No gross nor histologic changes were detected in tissues of the control.

### 5.4. Discussion

All infected suckling calf-camels did not show clinical signs, although *Toxoplasma gondii* tachyzoites were detected in the heart, lungs and kidneys of all infected calf-camels. *Toxoplasma gondii* cyst was detected in the brain of suckling calf-camel (C).
Figure (27): A photomicrograph of *Toxoplasma* cysts in a brain of suckling calf-camel (H & E)x400. Notice the lymphoid cells infiltration.
Clinical signs depend on the number of tachyzoites released, the ability of the host immune system to limit the spread of tachyzoites (humoral antibody responses and cell-mediated immunity may control tissue-cyst formation in host’s neural and muscular tissues) and the organs damaged by these tachyzoites (Aiello, 1998).

The excretion of *Toxoplasma gondii* in milk of camels was studied by inoculation of mice with milk of the three experimentally infected she-camels.

Six mice inoculated with milk of she-camel (a) & (c) became infected and non of the three mice inoculated with milk of she-camel (b) became infected.

*Toxoplasma* antibodies were detected in sera of all infected suckling calf-camels with low antibody titers. This may reveal that the concentration of *Toxoplasma gondii* tachyzoites in milk of camel was low.

This results were found in agreement with those of Dubey (1980) who found that the chance of *Toxoplasma gondii* being in the milk of naturally infected goats is very small.
CHAPTER VI

The effect of acute sarcocystosis on calf-camels
6. The effect of acute sarcocystosis on calf-camels

6.1. Introduction

Several species are pathogenic to intermediate hosts; the pathogenicity is of different gravity including a possible fatal outcome of the host. Acute sarcocystosis is caused by schizogonous phases in vascular endothelium or other cells of various organs, while the chronic disease results from sarcocyst formation in brain, striated or cardiac musculature of intermediate hosts. The principle pathogenic effect in the intermediate host is attributed to the second stage of merogony in the vascular endothelium. Experimental infection of calves with *Sarcocystis* has resulted in mortality (Johnson *et. al.*, 1975). Goats experimentally infected with *Sarcocystis* showed anaemia, weight loss, and death (Dubey *et. al.*, 1981).

A high prevalence of *Sarcocystis* in camels was reported, 81% in the Sudan (Hussein and Warrag, 1985), 82.5% in Somalia (Borrow *et al.*, 1989) 88.35% in Suadi Arabia (Fatani *et. al.*, 1996), 91.6% in Iraq (Latif *et al.*, 1999) and 45.5% in Ethiopia (Woldemeskel and Gumi, 2001). However little is known regarding the effect of *Sarcocystis* infection on camels.
The purpose of this study was to determine the pathogenicity of sarcocystosis on camels given different doses of *Sarcocystis* sporocysts.

**6.2. Materials and Methods**

Four naïve calf-camels (4-6 week-old) were obtained from camels herder at the outskirts of Omdurman. The animals were kept in animal pens at the Central Veterinary Research Laboratories.

Each calf-camel was inoculated orally with $1 \times 10^2$, $1 \times 10^5$ or $1 \times 10^6$ *Sarcocystis* sporocysts (chapter II section 2.9). The *Sarcocystis* sporocysts were isolated from puppies that were fed on camel meat as described in chapter II section 2.4 and section 2.7. One calf-camel was not infected and left as control.

During the experimental period, the animals were fed Alfa hay and concentrates.

Blood samples were taken once a week from the jugular vein using heparinized vacutainer tubes (Becton-Dickinson, France) for haematological studies as described in chapter II section 2.15.

Tissue samples from different organs of the calf-camels were fixed in neural buffered 10% formal saline solution for histopathological studies as described in chapter II section 2.11.
6.3. Results

Calf-camel I inoculated with 1X10^6 sporocysts went off food 14 days after infection (DAI). It became anaemic, lethargic, weak and remained so until it died 21 days after infection.

The red blood cell RBC counts decreased from 8.1X10^{12}/L to 3.6X10^{6}/L (Fig. 28). The hemoglobin concentration fell from 9.5g/dl to 5.0g/dl(Fig. 29). The initial PCV value of 0.290L/L fell to 0.150L/L (Fig. 30).

No significant gross lesions were seen at postmortem examinations. Microscopic examination of tissues revealed thrombus, haemorrhages and intense infiltration of lymphoid cells in lungs (Fig. 31) and mesentric lymph nodes (Fig. 32). The kidneys revealed glomereolonephritis associated with hemorrhage and infiltration of lymphoid cells. Haemorrhages were also found in the brain, heart, skeletal muscles and intestine.

Calf-camel II inoculated with 1x10^5 Sarcocystis sporocysts became weak, anaemic and off food 22 days after infection. It died 27 DAI. The RBC count decreased from 7.9x 10^{12}/L to 4.7 x10^{12}/L (Fig. 28). The hemoglobin concentration fell from 10.0 g/dl to 6.0 g/dl (Fig. 29). The initial PCV value of 0.300 L/L fell to 0.180 L/L (Fig. 30). Postmortem examination revealed petichial and ecchymotic hemmmorrhage in the serosa of the entire gastrointestinal tract; enlargement of mesentric lymph nodes were associated with oedema.
Figure (28):

RBCS values of calf-camels infected with *sarcozystis*
Figure (29): Haemoglobin g/dl for calf-camels infected with *Sarcocystis*
Figure (30) :

PCV values of calf-camels infected with Sarcocystis

PCV value/LL

Time before and after infection
Figure (31): A histosection in lung of calf-camel I (H & E)x100. Notice haemorrhage.

Figure (32): A histosection in lymph node of calf-camel I (H & E)x100. Notice haemorrhage and necrosis.
and hemorrhage. The abdominal and thoracic cavities contained excessive serosanguinous fluid. Microscopic examination revealed hemorrhage, oedema and mild infiltration of inflammatory cells in the heart. Development of schizonts occurred within endothelial cells of blood vessels of esophagus, diaphragm and tongue. The spleen, lymph nodes, and intestines were hemorrhagic, edematous and congested. were seen in the lung (Fig. 33). Liver showed vacuolation of cells heamorrhages and congestion (Fig. 34).

The calf-camel III infected with $1 \times 10^2$ sporocysts survived 74 days after infection when it became weak, off food and then died 90 days after infection. The RBC count decreased from $8.7 \times 10^{12}/L$ to 6.9 g/dl (Fig. 28). Hemoglobin concentration fell from 9.7 g/dl to 8.0 g/dl (Fig. 29). The initial PCV value of 30 L/L fell to 24 L/L (Fig. 30).

No significant gross lesions were seen at postmortem examinations.

Microscopic examination revealed the presence of *Sarcocystis* cysts located lengthwise between the muscle fibers of heart, oesophagus, tongue, diaphragm and all skeletal muscles examined (Fig. 35). The sarcocysts were spindle-shaped measured 70-130 µm x 20-25 µm and had a thick cyst wall 2 µm in diameter. The cyst wall divided the cyst into compartments and it was formed of two layers; an outer thick layer and a smooth inner one.
Figure (33): A histosection in lung of calf-cael II (H & E)x400. Notice oedema and lymphoid cells.

Figure (34): A histosection in liver of calf-camel II (H & E)x100. Notice vacuolation of hepatocytes.
Immature *Sarcocystis* cysts were found in the brain (Fig. 36). Lung showed thrombus, emphyzema and infiltration of mononuclear cells (Fig. 37). Liver showed vaccul ation of cells, haemorrhages and infiltration of mononuclear cells (Fig. 38).

**6.4. Discussion**

In this study clinicopathological picture reported in Sarcocystosis in experimentally infected camels is similar to the disease occurred in cattle, sheep and goats (Johnson, *et. al.*, 1975; Leek *et. al.*, 1977; Dubey, *et. al.*, 1981).

First generation meronts developed in aTRIES and arterioles up to 16 DAI, were apparently non pathogenic. The cattle and sheep inoculated with *Sarcocystis* did not die or became sick during this phase.

Calf-camel I died 21 DAI and calf-camel II died 27 DAI. This coincided with the development of second generation meronts (Johnson *et. al.*, 1975).

The release of merozoites in the infected host may be a factor in the development of anaemia (Dubey *et. al.*, 1981).

The development of haemorrhages in many organs is a common feature of Sarcocystosis in sheep, goats and cattle which were experimentally infected.
Figure (35) : A photomicrograph of *Sarcocystis* cyst between muscle fibers of calf camel III (H & E)x400

Figure (36) : A photomicrograph of immature *Sarcocystis* cyst in brain of calf-camel III (H & E)x400.
Figure (37) : A histosection in lung of calf-camel III (H & E)x100. Notice thrombus in blood vessel.

Figure (38) : A histosection in liver of calf-camel III (H & E)x100. Notice vacuolation and necrosis of hepatocytes.
Calf-camel III inoculated with a few number of *Sarcocystis* sporocysts survived the acute stage of sarcocystosis and died 90 days after infection. The death may be due to the sarcocystin toxin which was released from *Sarcocystis* cysts and affected the central nervous system, heart, lung, liver and intestine.

*Sarcocystis* tissue cysts detected in the calf-camel III were similar to those reported by Hilali and Mohamed (1980), Manal (1995).
CHAPTER VII

General Discussion
7. General Discussion

This study revealed a widespread prevalence of *Toxoplasma* antibodies among Sudanese camels (61.7%). El Gedarif shows the highest prevalence rate (68.3%).

A prevalence of 64.6% was detected when Butana, El Gedarif and El Shawak were considered as one study area. This finding was nearly similar to that of El amin *et. al.* (1992). Who reported a prevalence of 67% in Butana plains.

A prevalence of 59% was detected when Kordofan and El Hamra were considered as one study area, while a prevalence of 51% was detected in Nile River.

Camels under one year of age have a high titer at 1 : 1024, this may be due to a congenital infection. A highly significant difference between the sero-prevalence in the different age groups in the total samples was established (P<0.01). This revealed a high prevalence among old animals. It is conceivable that the longer an animal lives, the greater the chance of it being exposed to *Toxoplasma* oocysts.

The morphological and biological characteristics of the isolated oocysts of *Toxoplasma gondii* were similar to the description given by Frenkel *et. al.*, (1970); Sheffield and Melton (1970).
Toxoplasma gondii infection was confirmed by the serological tests done on camels and mice, by the detection of Toxoplasma tachyzoites in tissues of newly borne and suckling calf-camels. The detected cysts in calf-camels and the brains of mice were similar to the description given by Dubey (1977).

Acute acquired toxoplasmosis is most commonly assymptomatic. The host immunologic response to initial acute infection plays a principle role in the appearance of clinical symptoms.

This study revealed a relationship between the time of exposure of pregnant she-camels to Toxoplasma gondii infection and the pathogenicity on their offspring; the effect of Toxoplasma gondii on calf-camel 1 (infection at the first half of pregnancy) was more severe than the effect of Toxoplasma infection on calf-camel 2 (infection at the second half of pregnancy) although the infective dose was the same (1x10^5 sporulated T. gondii).

On the other hand calf-camel 3 was born a full-term dead while calf-camel 4 was born weak (the infective dose was 1x10^6 sporulated Toxoplasma oocysts).

These findings were similar to those of Dubey (1981b) who found that goats experimentally infected during the first months of pregnancy delivered dead lambs, whereas the damage on lambs was
less severe when ewes were infected during the later stage of pregnancy. Although Astrid et. al. (2000) found that the risk of symptomatic congenital toxoplasmosis and the severity of the disease are inversely related to the stage of gestation in which transmission occurs.

Foetal brains and eyes were the most affected organs in all experimentally infected calf-camels.

Diarrhoea was the main clinical symptom that was found in all calf-camels. *Toxoplasma gondii* schizonts, congestions, haemorrhages and infiltration of lymphoid cells (esenophils cells, lymphocytes) were detected microscopically in the intestinal villi. Esenophilic reaction was a result of the parasitic infection.

*Toxoplasma gondii* tachyzoites were not isolated from mice inoculated with pepsin digest, this may reveal the absence of well developed *Toxoplasma* cyst in the calf-camel tissues.

*Toxoplasma gondii* tachyzoites were not demonstrated in histologic section of placentae, but isolated from mice inoculated with those placentae, this may be due to the presence of low number of tachyzoites in placentae.

The clinicopathological picture of infected placentae is similar to those given by Dubey (1981a).
This study confirmed the excretion of \textit{Toxoplasma gondii} tachyzoites in milk of camels. Jackson, Hutchison (1989) found \textit{Toxoplasma gondii} in the milk of sheep and cows. Sacks \textit{et. al} (1982) detected tachyzoites of \textit{T. gondii} in milk of goats, while Bonametti \textit{et. al.} (1997) reported the presence of \textit{Toxoplasma gondii} tachyzoites in the milk of a woman.

Suckling calf-camels acquired toxoplasmosis from milk of their infected mothers. Histologic examinations revealed the presence of \textit{Toxoplasma} cysts in the brains of suckling calf-camels and the brains of mice that were inoculated with milk of infected she-camels.

The concentraion of \textit{Toxoplasma gondii} tachyzoites excreted in milk of infected she-camels was low, although the camels received a high dose of infective \textit{Toxoplasma gondii} oocysts. This was shown by the low titers of \textit{Toxoplasma} antibodies detected in the suckling calf-camels sera and by the degree of mice infection; mice inoculated with milk from she-camel (a) & (c) became infected while those inoculated with milk from she-camel (b) became uninfected.

Dubey (1980) reported that \textit{Toxoplasma gondii} tachyzoites were excreted in the milk of goats in low concentration.
In this study the high seroprevalence of *Toxoplasma gondii* reported in pastoral camels in the Sudan may be of public health significance, since nomads consume milk of camels raw.

Although tachyzoites are sensitive to proteolytic enzymes and are destroyed by gastric digestion, a recent study showed that tachyzoites survived for up to two hours in acid pepsin solutions, and that oral application of tachyzoites might have caused an infection (Dubey, 1998a).

Rieman *et. al* (1975); Sacks *et. al*, (1982) suggested that tachyzoites may enter the host by penetration of mucosal tissue and thereby gain access to the host’s circulation or lymphatic system before reaching the stomach.

Calf-camels infected orally with different doses of *Sarcocystis* sporocysts showed different gravity of infection; calf-camels inoculated with $1 \times 10^6$ and $1 \times 10^5$ died 21 days after infection and 27 days after infection respectively, calf-camel inoculated with $1 \times 10^2$ died 74 day after infection.

The clinicopathological picture reported in sarcocystosis in calf-camel I and calf-camel II is similar to the one seen in cattle, sheep and goats (Johnson *et. al.*, 1975; Leek *et. al.*, 1977; Dubey *et. al.*, 1981) respectively.
The development of hemorrhages in many organs is common in sarcocystosis of sheep, goats and cattle. First generation meronts of *Sarcocystis* develop in arteries and arterioles up to 16 days after infection, and are considered non pathogenic; cattle and sheep inoculated with *Sarcocystis* do not die or become sick during this phase.

Clinical signs of acute sarcocystosis are associated with the development of the second generation meronts in capillary endothelial cells (Leek and Fayer, 1978).

In this study calf-camels I and II died 21 DAI & 27 DAI respectively and this may be due to the development of second generation meronts.

Calf-camel III inoculated with $1 \times 10^2$ sarcocystis sporocysts survived the acute stage and died 90 days after infection. The death may be due to the sarcocystin toxin which was released from *Sarcocystis* cysts and affected the central nervous system, heart, lung, liver and intestine.

The release of merozoites or their metabolites in the infected host may be a factor in development of anaemia (Dubey *et. al.*, 1981).
The morphology of *Sarcocystis* tissue cysts detected in calf-camel III was similar to that detected by Hilali and Mohamed (1980); Manal (1995).

*Toxoplasma gondii* has developed a broad range of potential routes of transmission. It is currently not known which of the various routes of transmission is more important epidemiologically. However, the prevalence of *Toxoplasma gondii* infection is not confined to the presence of a certain host species. Its life cycle may continue indefinitely by transmission of tissue cysts between intermediate hosts and also by transmission of oocysts between definitive hosts.

Recognition of transplacental toxoplasmosis in camels is important from economic and public health points of view. Cats may eat infected placentae, a single infected cat may shed more than 100 millions of oocysts in its feces (Jackson, Hutchison 1989).

Under environmental conditions with sufficient aeration, humidity and warm temperature, oocysts sporulated and became infectious within 1-5 days (Evans, 1992). Sporulated oocysts of *Toxoplasma gondii* are very resistant to environmental conditions and remain infectious in moist soil or sand for up to 18 months (Frenkel, 2000). Longevity of the infectious oocysts under conditions similar to those in which cats bury their feces is of major importance in the transmission of Toxoplasmosis for livestock.
Oocysts are distributed in the environment through wind, rain and surface water (Astrid et. al., 2000). Hay, straw and grain which had been contaminated with cat faeces may be sources of infection for livestock.

There are many factors that have an impact on the epidemiology of *Toxoplasma gondii* infection of camels, such as the type of camel management, the density of cats or wild felines in the environment, the environmental conditions that have an influence on the sporulation of oocysts, the hygienic standards of abattoirs as well as the different habits of human consumers.

Environmental stress do not affect sporulation of *Sarcocystis spp.* As sporocysts are fully infectious when passed in faeces. Transport hosts can protect and disseminate sporocysts in the environment.

To control Toxoplasmosis and sarcocystosis in intermediate hosts by management is impractical in wildlife populations. However, in livestock operation, management can reduce sporocysts contamination of feed and water supplies. Complete elimination of infection is probably impossible. To reduce infection, carnivores and felids should not be allowed scavenge on livestock carcasses. Carcasses and affals of livestock should be buried or incinerated. Although these management techniques are simple they may be difficult to implement.
References


