Epidemiological study of *Cryptosporidium* at the wildlife-livestock and human interface in the western boundaries of the Kruger National Park

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*Cryptosporidium* spp.

- Protozoan parasite
- Replicates in the small intestine of a large number of vertebrates (mammals, birds & reptiles)
Main source of infection: contaminated environment by faeces

- Oocysts: immediately infectious when excreted
- Very stable & survive up to 6 months in a moist & cool environment
- Unaffected by chlorine or other disinfectants added to water

Cryptosporidium spp.

Phylum: Apicomplexa

16 species:
- C. andersoni
- C. baileyi
- C. bovis
- C. cervine*
- C. canis*
- C. felis*
- C. galli
- C. hominis*
- C. melagridis*
- C. molnari
- C. muris*
- C. parvum*
- C. saurophilum
- C. serpentis
- C. suis*
- C. wrairi

* * Cryptosporidium spp identified in human
* * Known to be of major zoonotic importance
**Cryptosporidium** spp. in humans & animals

- Significant morbidity & mortality in young & immunocompromised individuals:
  - **Human** - among young children causes 45% of diarrheal deaths in Bangladesh, Brazil & in several African countries
  - **Human** - life-threatening diarrhoea in HIV positive patients & therefore rural population in southern Africa with the highest prevalence of HIV/AIDS worldwide, are particularly at risk
  - **Livestock** - important cause of diarrhoea in calves (1-3 week); adults shed the parasite without symptoms
  - **Wildlife** – identified as reservoirs; infection appears to be asymptomatic

**Cryptosporidium** spp. in humans & animals

No specific treatment available:

- **Livestock** - in calves quinazolina used in some european countries; its efficacy has not been demonstrated
- **Humans** – no drug effectively treats cryptospoiriosis; only reduces disease severity in some cases
Prevalences of *Cryptosporidium* in Africa

**In Wildlife**
- Tanzania: African Buffalo: 22%
- Zebras: 28%
- Wildebeest: 27%
- South Africa: No data

**In Livestock**
- Tanzania: 5.3% in calves
- Uganda: 38% in calves
- Zambia: 19.2% in calves
- South Africa: No data

**In Human**
- Uganda: 25% in diarrhoeic children
- Guinea Bissau: 7% in children
- Tanzania: 17.3% in HIV patients
- South Africa: 18% school children & Hospital patients (Samie et al., 2006)

**Objective**

- Determine the zoonotic importance of *Cryptosporidium* spp. at the wildlife, livestock & human interface in KNP

- Understand the epidemiological patterns of *Cryptosporidium* spp. among the different compartments (wildlife, livestock and human)
Research Questions

1. Are wildlife species in KNP a reservoir of Cryptosporidium?
2. What is the prevalence of Cryptosporidium in livestock adjacent to KNP?
3. Is Cryptosporidium a neglected zoonosis in human communities living close to the KNP?
4. What are the epidemiological patterns of circulation and transmission of Cryptosporidium at the wildlife/livestock/human interface in KNP?

Wildlife: Study Area

Kruger National Park
- Tshokwane (A), distant from KNP fence
- Skukuza (C), adjacent to fence

Private game reserve
- Sabi Sand (B), adjacent to fence
Wildlife: Material & Methods

Collection of faecal samples from wildlife:
- Three of the most commonly seen species
- Collected during dry & rainy seasons 2008/2009

<table>
<thead>
<tr>
<th></th>
<th>Skukuza</th>
<th>Sabie Sand</th>
<th>Tshokwane</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo</td>
<td>103</td>
<td>71</td>
<td>92</td>
<td>266</td>
</tr>
<tr>
<td>Elephant</td>
<td>90</td>
<td>76</td>
<td>90</td>
<td>256</td>
</tr>
<tr>
<td>Impala</td>
<td>98</td>
<td>92</td>
<td>90</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>291</td>
<td>239</td>
<td>272</td>
<td>802</td>
</tr>
</tbody>
</table>

Wildlife: Material & Methods

Laboratory analysis:
- Immunofluorescent antibody (IFA) commercial kit (anti- Cryptosporidium parvum monoclonal antibody technique)
- Ziehl Neelsen (ZN) – detects all Cryptosporidium spp.
- Confirmation of Positives ZN & IFA with RT-PCR
## Wildlife: Results

### Prevalence with Ziehl-Neelsen staining

<table>
<thead>
<tr>
<th></th>
<th>Skukuza</th>
<th>Sabi Sand</th>
<th>Tshokwane</th>
<th>Total/species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elephant</strong></td>
<td>34.4%</td>
<td>35.7%</td>
<td>6.6%</td>
<td>25.8%</td>
</tr>
<tr>
<td></td>
<td>(12/35)</td>
<td>(10/28)</td>
<td>(2/30)</td>
<td>(24/93)</td>
</tr>
<tr>
<td><strong>Buffalo</strong></td>
<td>3.2%</td>
<td>6.9%</td>
<td>6.6%</td>
<td>5.5%</td>
</tr>
<tr>
<td></td>
<td>(1/31)</td>
<td>(2/29)</td>
<td>(2/30)</td>
<td>(5/91)</td>
</tr>
<tr>
<td><strong>Impala</strong></td>
<td>3.2%</td>
<td>5.9%</td>
<td>3.5%</td>
<td>4.2%</td>
</tr>
<tr>
<td></td>
<td>(1/31)</td>
<td>(2/34)</td>
<td>(1/29)</td>
<td>(4/94)</td>
</tr>
</tbody>
</table>

Overall prevalence 11.8%

## Wildlife: Results

### Prevalence with direct immunofluorescent antibody test (IFA)

<table>
<thead>
<tr>
<th></th>
<th>Skukuza</th>
<th>Sabi Sand</th>
<th>Tshokwane</th>
<th>Total/species</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elephant</strong></td>
<td>8%</td>
<td>0%</td>
<td>4%</td>
<td>4.2%</td>
</tr>
<tr>
<td></td>
<td>(4/50)</td>
<td>(0/44)</td>
<td>(2/50)</td>
<td>(6/144)</td>
</tr>
<tr>
<td><strong>Buffalo</strong></td>
<td>2%</td>
<td>2.5%</td>
<td>0%</td>
<td>1.4%</td>
</tr>
<tr>
<td></td>
<td>(1/50)</td>
<td>(1/40)</td>
<td>(0/50)</td>
<td>(2/140)</td>
</tr>
<tr>
<td><strong>Impala</strong></td>
<td>0%</td>
<td>5%</td>
<td>0%</td>
<td>1.8%</td>
</tr>
<tr>
<td></td>
<td>(0/50)</td>
<td>(3/59)</td>
<td>(0/50)</td>
<td>(3/161)</td>
</tr>
</tbody>
</table>

Overall prevalence 2.5%
Results wildlife

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>OR</th>
<th>95% C.I. (OR)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>Tshokwane</td>
<td>1*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sabi Sand</td>
<td>3.6</td>
<td>1.2;11.1</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Skukuza</td>
<td>2.9</td>
<td>1.0; 8.8</td>
<td>0.057</td>
</tr>
<tr>
<td>Species</td>
<td>Impala</td>
<td>1*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Buffalo</td>
<td>1.3</td>
<td>0.3; 5.2</td>
<td>0.665</td>
</tr>
<tr>
<td></td>
<td>Elephant</td>
<td>8.4</td>
<td>2.8; 25.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

- Prevalence was significantly higher in elephant than other species.
- Prevalence was higher in areas close to the fence

Wildlife: Main Conclusions

- *Cryptosporidium* spp. are present in KNP wildlife
  - Potential source of infection to livestock & human at the interface?
  - Is wildlife close to the fence more exposed?

- Prevalence might be higher than the one observed
  - Low sensitivity & specificity of ZN
  - Samples may contain genotypes that could not be detected
  - Analyzed samples: collected during dry season
Wildlife: Way forward

• Detection of all Cryptosporidium spp circulating in the wildlife population: PCR, genotyping & subtyping

• Analysis of seasonal variations (only dry season samples have been analyzed)

Livestock: Study Area

Bushbuckridge: 10 diptanks (●) located in close proximity to the fences of KNP & Sabi Sand
Livestock

- 1000 samples randomly selected
- Collected in rainy and dry season
- All ages
- immunofluorescent antibody (IFA) commercial kit (anti- Cryptosporidium parvum monoclonal antibody technique)

Results from dry season
- Overall prevalence: 1% (3/300)

Livestock: Main Conclusion

Low prevalence in cattle (1%):
- IFA test specific for the detection of Cryptosporidium parvum oocysts (species of major zoonotic importance)
- Other Cryptosporidium spp. might circulate in cattle
Livestock: Way forward

• PCR, genotyping & subtyping to detect and characterize Cryptosporidium strains in the cattle population.

• Analysis of seasonal variations (only dry season samples have been analyzed)

Human communities: Study Area

7 clinics located in communities in close proximity to KNP:

- Belfast Clinic
- Justicia Clinic
- Lillydale Clinic
- Lillydale Private Hospital
- Agincourt Clinic
- Calcutta Clinic
- Oakley Clinic
Human communities: Way forward

• Collection of human faecal samples (approx. 200)

• PCR, genotyping & subtyping of human samples to detect the presence of Cryptosporidium and the different Cryptosporidium spp.

• Determine the zoonotic importance of Cryptosporidium spp from livestock and wildlife origin.

Expected Results

• Detect different Cryptosporidium spp. circulating in the wildlife, livestock & human population

• Establish links between Cryptosporidium spp. in humans, livestock & wildlife.

• Comparison of different diagnostic methods
Activities 2011

- **March 2011**: Collection of human faecal samples in the study area (7 clinics in Bushbuckridge).

- **April – August 2011**: PCR, genotyping & subtyping of Cryptosporidium of wildlife, cattle & human samples at National Center for Emerging and zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, USA

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Thank You