What we have learned from bovine tuberculosis research in the buffalo populations of the Kruger National Park

by

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Rationale for BTB research

- BTB is regarded as an alien disease (see KNP management objectives)
- BTB entered the KNP ecosystem relatively recently (history suggests late 1960's)
- Spread of disease within buffalo herds and spatial spread between herds has been faster than anticipated
- Bovine tuberculosis is a multi-host infection with slow progression of disease in the individual
- Its long-term impact on free-ranging wildlife populations with high infection rates is unknown
- Some TPC's have been developed, others still under discussion (Various aspects still need to be researched)

Research Progress

- **2000**
  Infection model, northern and far north buffalo BTB survey
- **2001**
  Boma BCG vaccine study
- **2002**
  Live capture of animals for research and disease-free breeding projects
- **2003**
  Northern and far north survey
- **2004**
  Semi free-range BCG study
- **2005**
  Southern lethal BTB survey
- **2006**
  Far north and Mozambique (LNP) BTB survey

DEVELOPMENT OF AN INFECTION MODEL WAS ESSENTIAL FOR EVALUATING VACCINE EFFICACY

- Intra-tonsilar challenge with virulent bacteria
- Repeatable & reliable method (Griffin *et al*, in red deer; Palmer *et al*, in cattle)
- 3 groups: control group, low dose (3×10^2cfu) and high dose group (3×10^4cfu)
- Infection result evaluated 22 weeks after challenge

Results of infection model

- Control group: No lesions
- Low dose group: 4/11 with lesions
- High dose group: 9/11 with lesions
- Histopathology resembled natural challenge
- Challenge dose had an influence on
  - Number of diseased animals
  - Severity of lesions
- Secondary spread of lesions – function of time or challenge dose?

Boma BCG vaccine study

- Used moderate dose of Bacille-Calmette Guérin (BCG) vaccine ($1.7 \times 10^5 – 1.3 \times 10^6$ cfu)
- Two groups: Control group and vaccinated group
- High challenge dose of live *M. bovis* (4×10^4cfu)
- Results evaluated 34 weeks after challenge

Results of boma BCG vaccine study

- BCG vaccine provided no significant protection against BTB
- Control group: 10/14 with lesions
- Vaccine group: 7/15 with lesions
- Foot and mouth (FMD) disease outbreak may have interfered with cellular immune response (lymphopaenia)
- Challenge dose had an influence on ??
  - Severity of lesions
  - Number of diseased animals
- Nine animals (5 control & 4 vaccinated) with secondary spread of lesions – linked to period of infection, dose at challenge or stress?

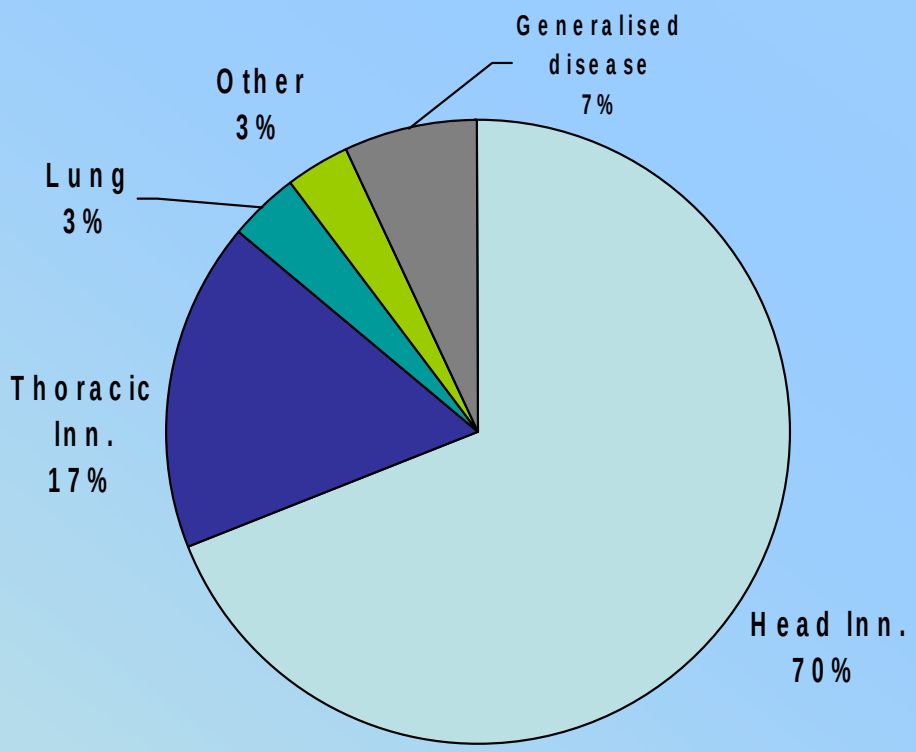
Semi free-range BCG vaccine study

- Four groups: Infected adult cows, calves, vaccinated yearling group & control yearling group
- Moderate dose of BCG vaccine (4.4×10^7 cfu)
- Initially one year contact period with the infected cows without BTB transmission occurring to the yearlings
- This was followed by artificially challenging both yearling groups with a low dose of virulent *M. bovis*
- Results evaluated 34 weeks after challenge

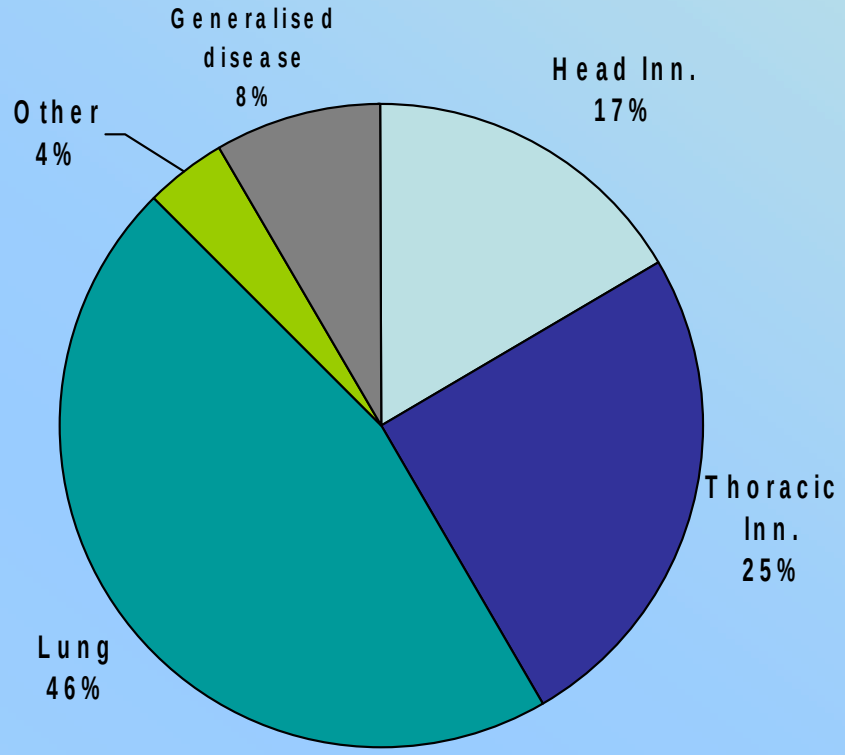
Results of semi free-range BCG vaccine study

- The BCG vaccine provided no significant protection against BTB
- Cows: 13/24 with lesions
- Calves: 2/9 with lesions
- Control group: 8/13 with lesions
- Vaccine group: 7/14 with lesions
- Very few males developed disease, whereas almost all yearling females were affected (stress related?)
- Secondary spread to thoracic organs in 3 animals in each of the yearling groups
- Secondary spread linked to infection period

Difference in lesion distribution between experimental and natural disease



Distribution of lesions in experimentally infected animals



Distribution of lesions in animals with natural disease transmission

Factors potentially having an influence on disease outcome and vaccine failure

- Infectious dose at challenge (live *M. bovis*)
- Route of challenge
- Repeat challenge events
- Age at vaccination
- Coincidental infections
- Stressful surroundings
- Exposure and sensitization to environmental mycobacteria

  M. terrae, M. vaccae, M. szulgai, M. kansasii, M. engbaekii, M. vanbaalenii, M. moriokaense, M. thermoresistibile & 3 unknown *mycobacterial spp*.

BTB monitoring surveys

- Types of surveys
  - Disease detection surveys

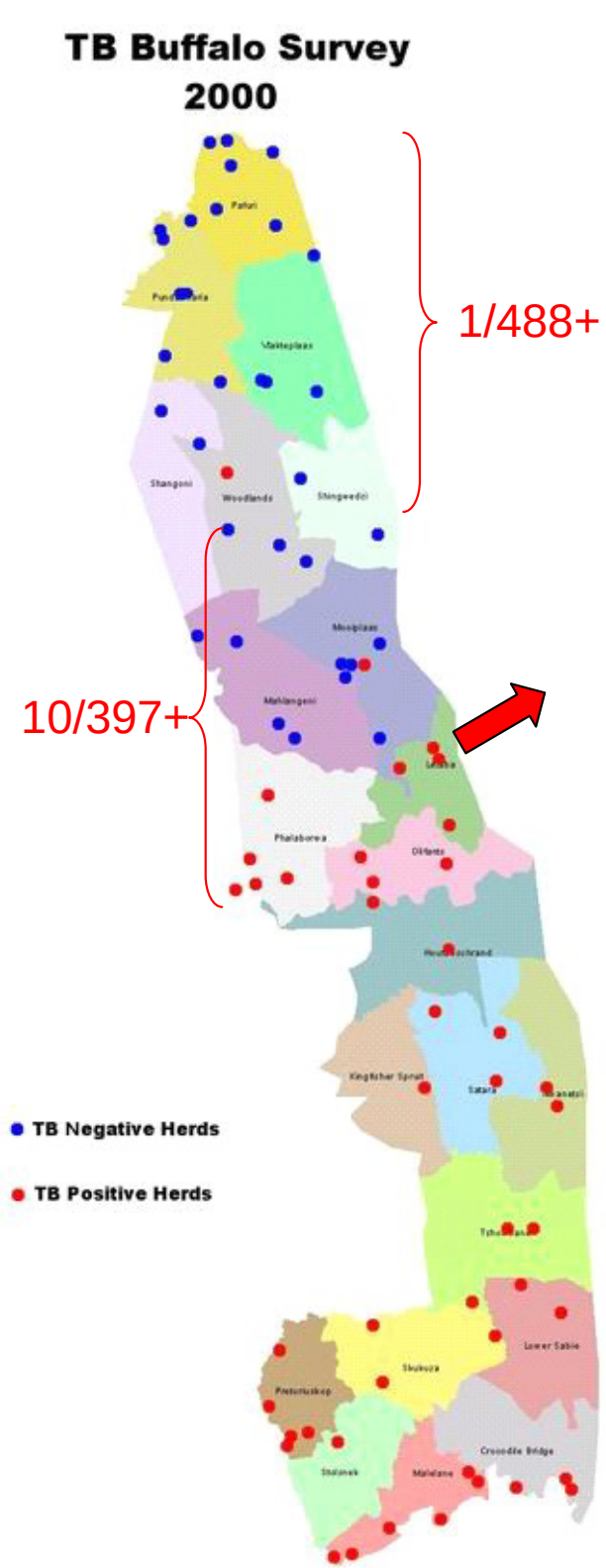
    KNP far north (2000) & LNP (2006)
  - Disease prevalence surveys

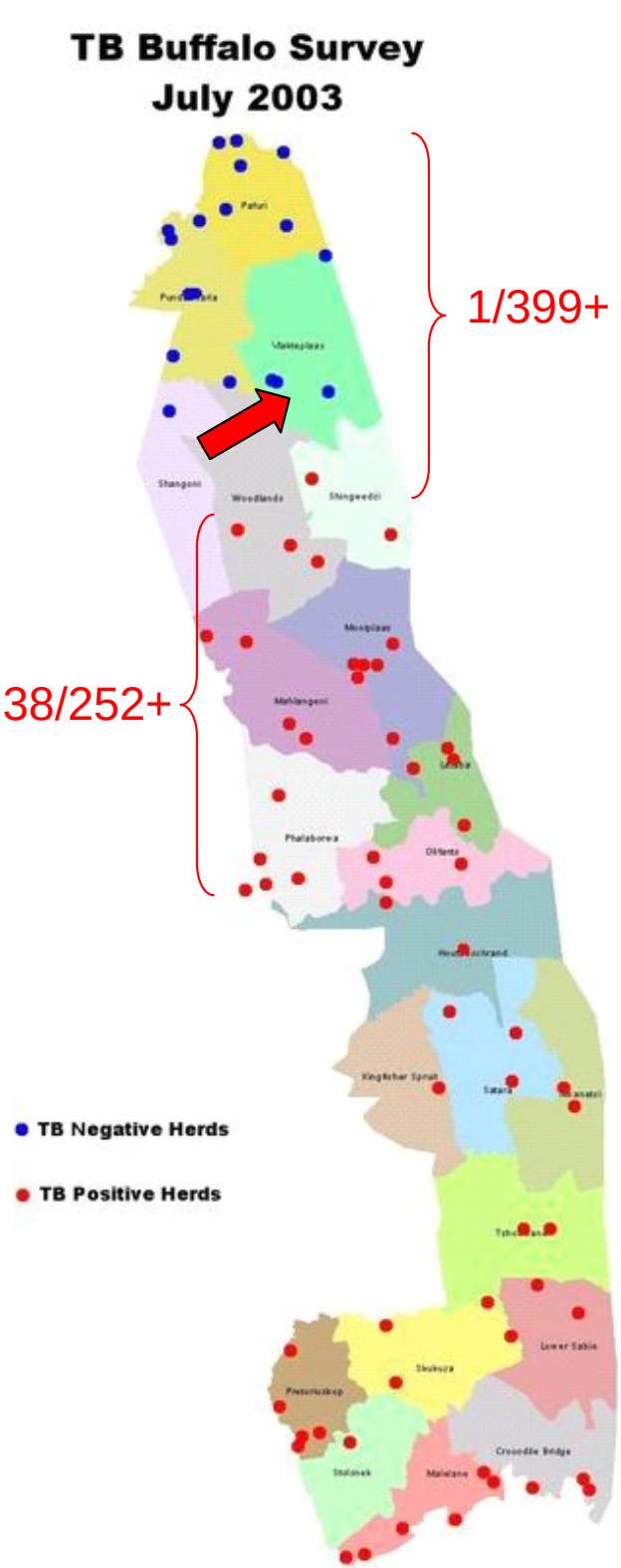
    KNP north (2000 & 2003), far north (2003) & south (2005)
- Sampling methods
  - Live capture: Using the interferon-gamma assay as the diagnostic test

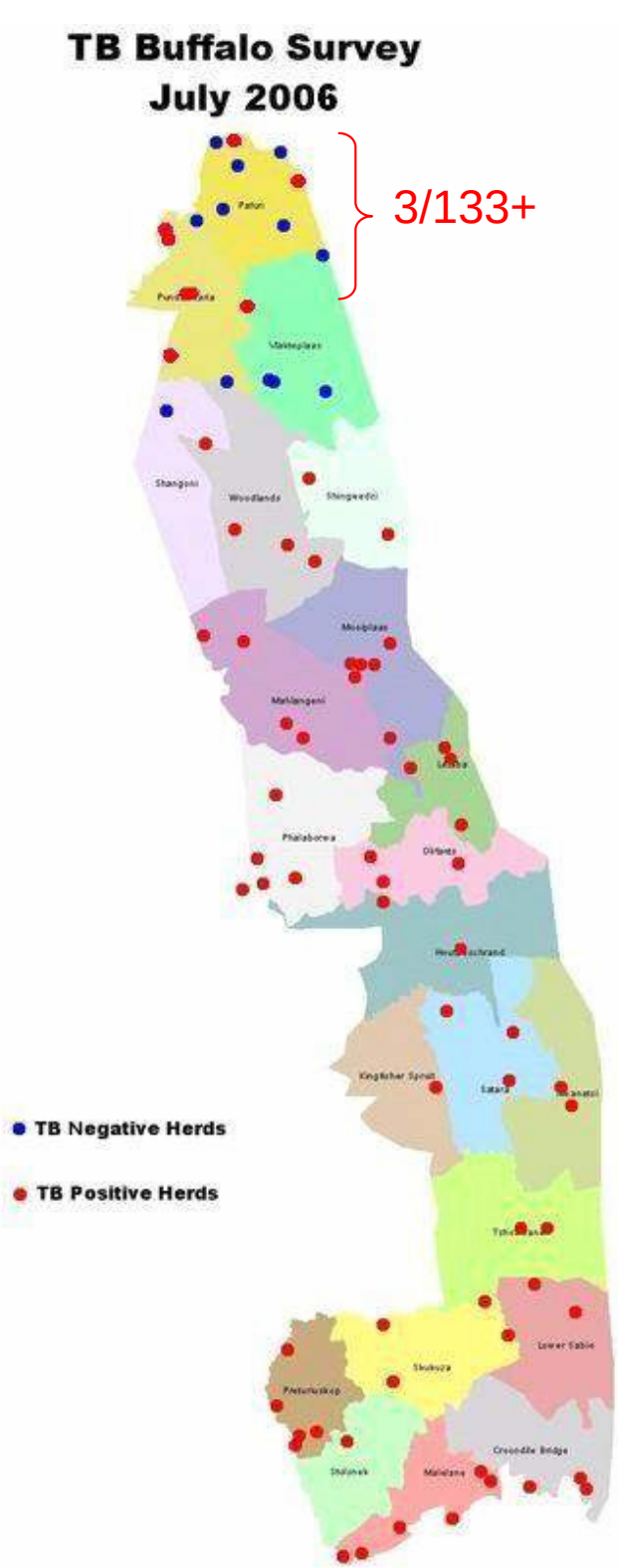
    KNP north & far north & LNP (Mozambique)
  - Lethal survey: Using identification of TB lesions during necropsy procedures, histopathology and culture as diagnostic tests

    KNP southern region

Results of live capture surveys


TB Buffalo Survey
2000
1/488+
10/397+
TB Negative Herds
TB Positive Herds


TB Buffalo Survey
July 2003
1/399+
38/252+
TB Negative Herds
TB Positive Herds


TB Buffalo Survey
July 2006
3/133+
TB Negative Herds
TB Positive Herds

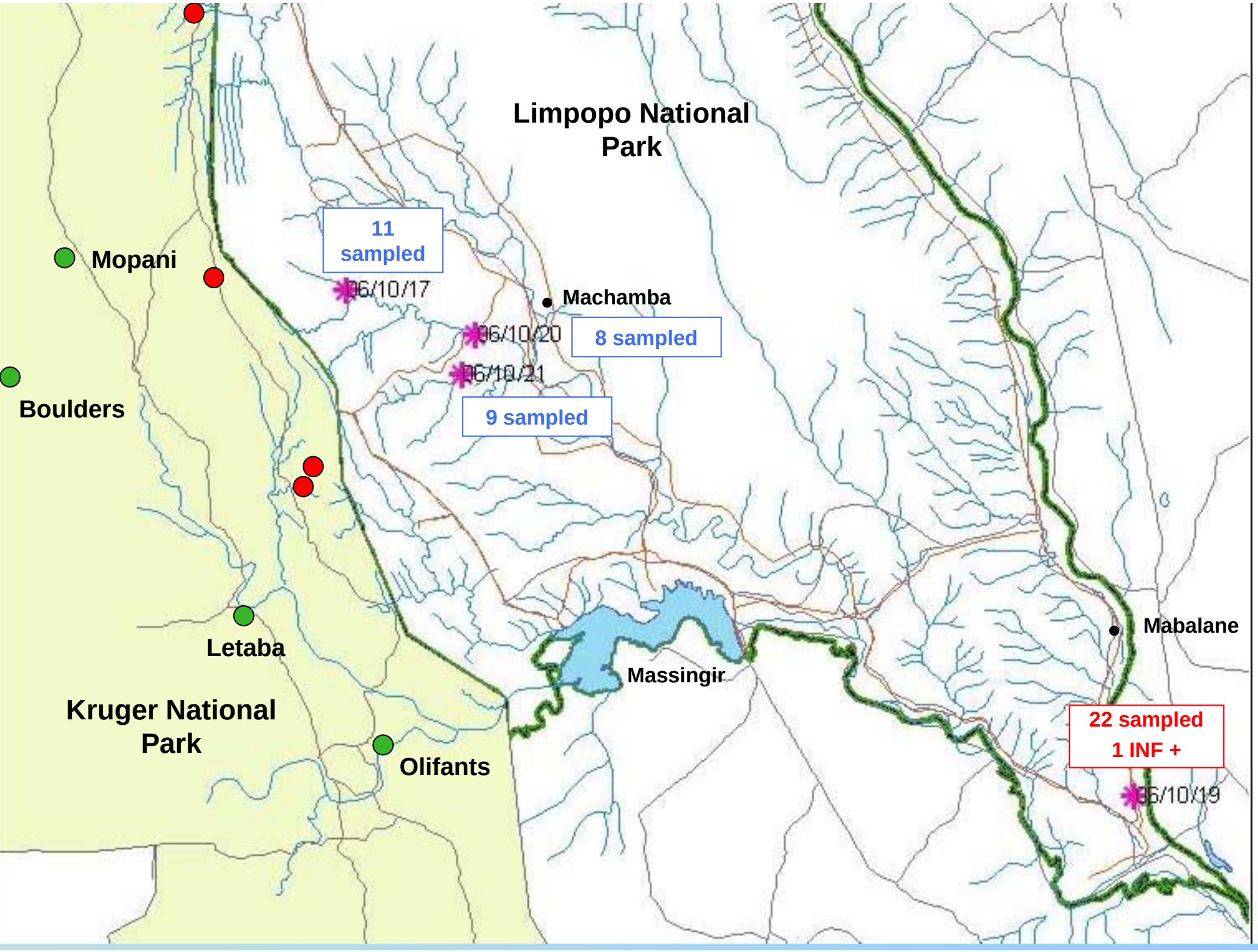
Results from KNP live capture surveys

- An increase in spatial distribution of disease was found during consecutive surveys
- An increase in disease prevalence was found during consecutive surveys

  North: From < 5% to 10 – 15%;

  Far north: From < 0.25% to < 0.5% to <5%
- Test positive animals that were euthanased were mostly sub-adult and adult females
- Bulls were rarely recaptured
- Necropsies on test positive animals revealed TB lesions in lungs, thoracic lymph nodes and sometimes head lymph nodes

Linking buffalo BTB surveys to TFCA neighbours (LNP Mozambique)


Limpopo National Park
11 sampled
06/10/17
Machamba
06/10/20
8 sampled
06/10/21
9 sampled
Mopani
Boulders
Letaba
Kruger National Park
Olifants
Massingir
Mabalane
22 sampled
1 INF +
06/10/19

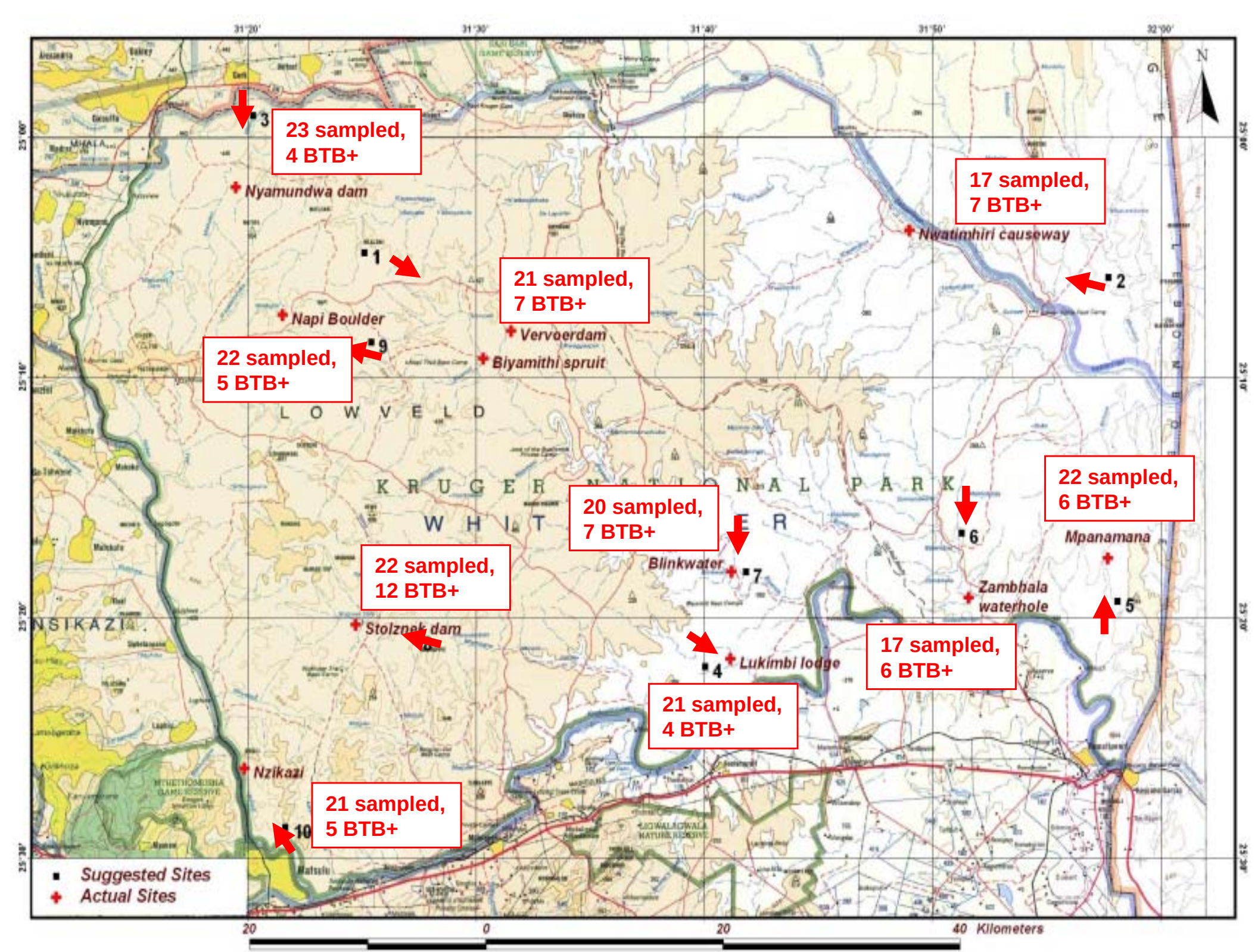
Results of survey in LNP

- Two distinct buffalo groups

  - Western group located in the Shingwedzi & Madonze drainages: total sample = 28

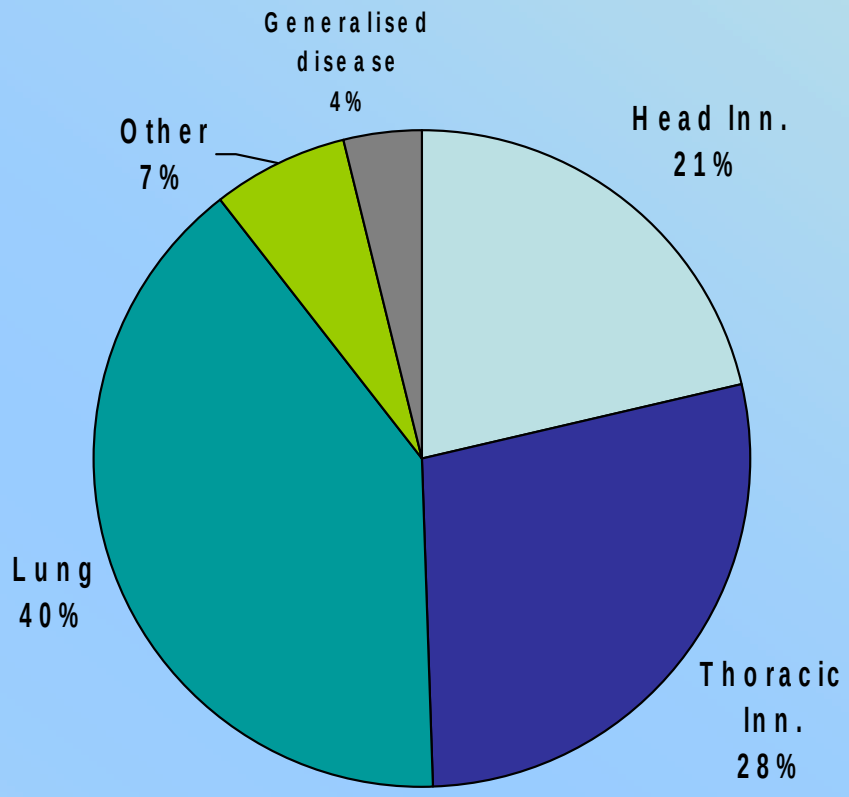
  - Eastern group near the confluence of the Olifants and Limpopo rivers: total sample = 22
- One false positive on gamma-interferon from eastern group – in contact with domestic cattle
- Western group – tests for FMD and Theileriosis revealed a similar disease pattern to KNP buffalo – majority positive
- Eastern group – isolated sub-population. A significant percentage of buffalo were negative for FMD and Theileriosis

Lethal survey of buffalo in southern region KNP (2005)

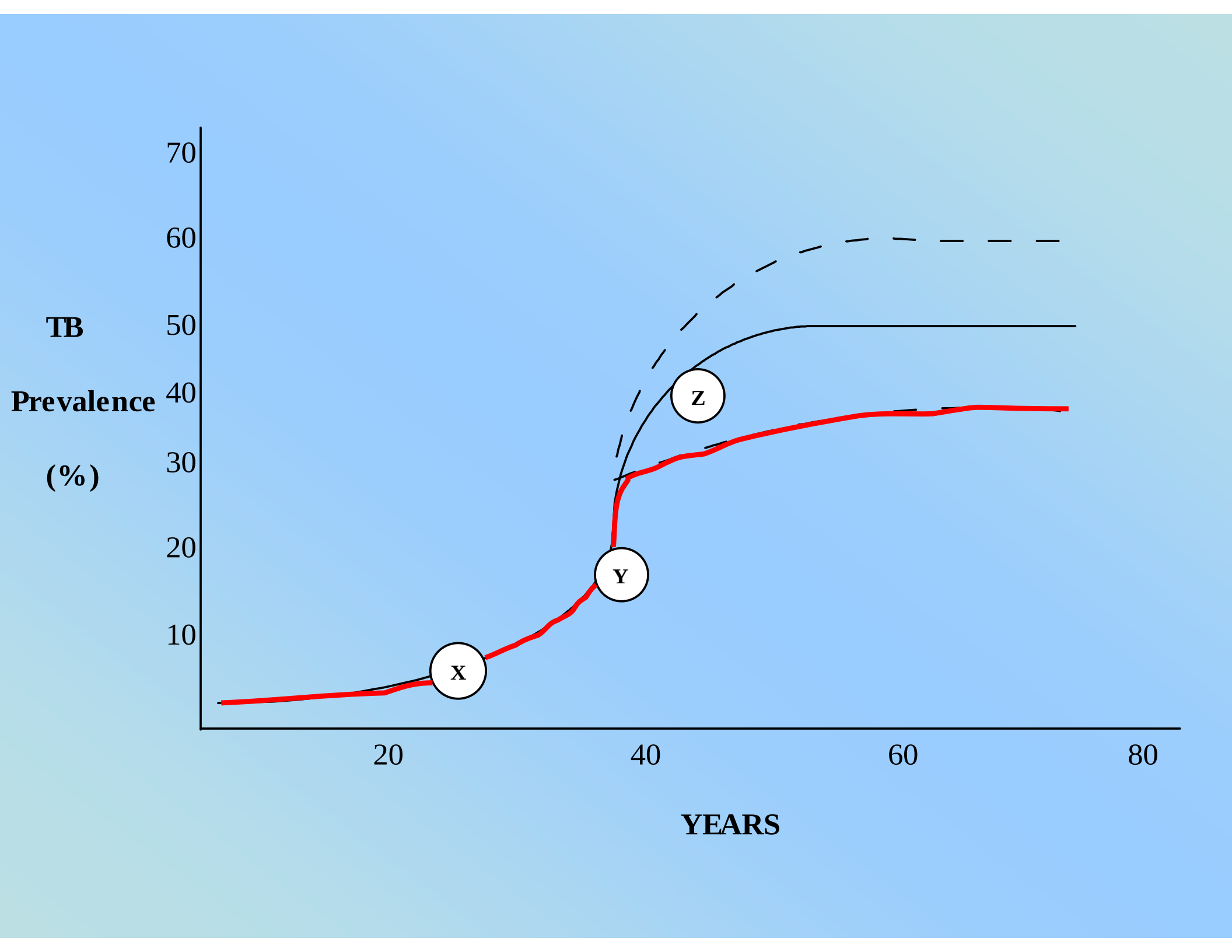

23 sampled,
4 BTB+
Nyamundwa dam
17 sampled,
7 BTB+
Nwatimhiri causeway
21 sampled,
7 BTB+
Napi Boulder
Vervoerdam
Biyamithi spruit
22 sampled,
5 BTB+
LOWVELD
KRUGER NATIONAL PARK
WHITE
20 sampled,
7 BTB+
22 sampled,
6 BTB+
Mpanamana
Blinkwater
Zambhala
waterhole
22 sampled,
12 BTB+
Stolznek dam
Lukimbi lodge
17 sampled,
6 BTB+
21 sampled,
4 BTB+
Nzikazi
21 sampled,
5 BTB+
Suggested Sites
Actual Sites
20 0 20 40 Kilometers

Results of the 2005 lethal survey

- The lesion distribution suggests that the main method of natural disease transmission remains aerosolized droplets of *M. bovis*
- Other surveys also indicated that test positive animals mainly had pulmonary lesions
- More females (42) than males (21) affected



Distribution of lesions in animals from the 2005 lethal sample


70
60
50
40
30
20
10
TB
Prevalence
(%)
X
Y
Z
20
40
60
80
YEARS

What does this all mean?

- There is a gradient of BTB infection in buffalo from south to north which translates into a similar gradient of infection pressure
- The risk of BTB transmission at interface areas appears to increase with higher disease prevalence in buffalo
- The risk of spill-over to other species appears to increase with increased disease prevalence in buffalo
- The long term impact on the buffalo population at sustained high prevalence is not yet known
- Census data of the last two years appears to indicate that the population growth of the southern buffalo populations is underperforming
- The effects of environmentally stressful conditions (e.g. drought) and other coinciding diseases might increase disease susceptibility, lesion progression and mortality index

So … where to now?

Recommendations

- Continued park-wide monitoring activities including interface areas are essential to understand disease dynamics and risks
- *Ad hoc* surveillance for BTB in all species should be continued
- Identifying and culling of animals with advanced BTB may reduce the number of super-shedders
- Continued evaluation of new vaccine candidates is essential
- New vaccines should be tested in younger animals
  (< 6 months)
  - still protected by maternal immunity to FMD
  - less sensitization from environmental exposure
- When evaluating vaccines, apply aerosol challenge – natural route
- Investigate genetic markers for resistance to intracellular bacteria.

Thank you !