

Research Proposal:

Improving Bovine Tuberculosis and Brucellosis Diagnostics in African Buffalo, Cattle and Lion

Establishment and optimization of single tube multiplex PCR for Mycobacterium bovis and Brucella abortus

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Research Period and Location:

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Introduction to Subject:

Bovine Tuberculosis (BTB) is most likely introduced in South Africa during the 18th century by European settlers with their imported cattle.³ It has been around in cattle ever since. The disease was first diagnosed in African Buffalo (*Syncerus Caffer*) in Kruger National Park (KNP) in 1990, but it is thought that it has been there for about 50 years.

The BTB-epidemic in South-African cattle and wildlife has several economic, environmental (regarding endangered species) and public health implications.³ It is thought the disease has negative long-term effects on population dynamics of African buffalo and spill-over hosts like lion, greater kudu, warthog and several other species. From an economic point of view BTB gives rise to national and international trade restrictions. Regarding the HIV epidemic among the human population of South-Africa and the wildlife-cattle-human interface, BTB i.e. its causal agent *M.Bovis*, is a serious zoonotic threat to human health, especially in immuno-compromised people.⁶

Bovine Tuberculosis is caused by *Mycobacterium bovis*, a member of the Mycobacterium Tuberculosis Complex (MTC). The MTC contains a variety of closely related mycobacteria, including *M.bovis*, *M.tuberculosis*, *M.africanum*, *M.canetti* and *M.necroti*.⁴ Tuberculosis is primarily an infection of the respiratory tract, where the agent uses the macrophage as primary host cell for intracellular replication.⁶ Phagocytosis following deposition of bacilli on the respiratory surface ensures interaction with the innate and acquired immune system. At first a cell-mediated immune-response (CMI) takes place, in a later stage of the disease B-cells are also involved.⁶

There are many tests available for the diagnosis of BTB. The most widely used is the tuberculin skin test, based on the CMI. A huge drawback for the use of this test in wildlife species is the fact that two sedations within a 2-3 days time interval are required. Besides that newly infected animals cannot be detected, positive results are primarily seen in animals infected for 1 to 9 weeks.² Main argument in favor of the use of the tuberculin skin test in cattle is its cost-effectiveness. More recently the IFN- γ test was introduced for in vitro diagnosis, also based on CMI. Two other tests, ELISA and FPA (fluorescent polarization assays), assess the humoral immune response. These are not adequate tools for detection of recently infected cattle, but they are good for detection of chronically infected animals. Detection of the causal agent in bacterial culture is considered the golden standard, whereas its DNA can also be detected by PCR. Finally post-mortem diagnosis (gross pathology and histopathology) can be made in the abattoir.

In the face of the many agro-economic, environmental and public health problems associated with BTB it is important to develop and validate diagnostic tools for cattle as well as different wildlife species, and ultimately develop a wildlife BTB vaccine.

Establishment and optimization of single tube multiplex PCR for *Mycobacterium bovis* and *Brucella abortus*

Introduction

Besides tuberculosis, brucellosis is thought to be highly prevalent in the Kruger National Park.¹ The causative agent for brucellosis is *Brucella abortus* (BA), which, like *M. bovis*, affects macrophages for replication.

Both agents have zoonotic potential and are thus of public health and economic importance. They pose a barrier for trade of animals and animal products and thereby also affecting translocation and breeding programs of endangered species.⁷

Symptoms of brucellosis in cattle are not pathognomic, bacteriological isolation and identification of *B. abortus* is considered the gold standard for definitive diagnosis. In cattle the diagnosis is generally based on serological tests like ELISA, Complement fixation (CFT), serum agglutination (SAT) or milk ring test (MRT). These tests indirectly determine the infective state of the host.⁵

Identification of *B. abortus* can also be done directly by PCR-assays, for example on blood, milk and lymph tissue. Several regions of the agents' genome have been described and used as targets for PCR (for example the IS711-genetic element, 16S-rRNA, 31 kDa omp, BCSP-31, 43 kDa omp and the omp2 gene).⁵

For BTB the skin test is used as main diagnostic tool in cattle, alternative tests like ELISA, FPA, IFN- γ test are useful but complementary to the skin test rather than real alternatives. For wildlife the skin test is not appropriate, as mentioned before. A specific and sensitive alternative is needed. PCR has proven to be a valuable technique for detection of *M. bovis* from biological samples like lymphoid tissue, milk and nasal swabs.⁸ Many species-specific markers have been identified in MPT40, oxyR and pncA genes. By using primers targeting these genes in PCR, *M. bovis* could be specifically identified, and thus differentiated from other mycobacteria. Also IS6110 is often used as target regarding PCR for MTC organisms.⁷

PCR of biological samples identifies animals actively shedding the organism; this is why caution is appropriate when interpreting PCR-results of chronically infected animals with granulomatous diseases like BTB, that are able to restrict shedding.⁷

Goals:

Both BTB and BA have been observed in African buffaloes and simultaneous infections may occur, therefore this species will be the main object of research. The proposed research aims at improving diagnostics by means of PCR for both diseases in this species. PCR-tests are available for *M. bovis* and *B. abortus* but need to be validated for use in buffalo lymphoid tissue samples. Furthermore a single tube multiplex PCR test is desired, incorporating both PCR-tests in one.

Materials and methods:

A number of frozen retropharyngeal lymphnode biopsies or their DNA-extracts from buffaloes with known BTB-status are available. So far there is no information about their BA-status.

Two conventional PCR-tests are available (for *M. bovis* and *B. abortus*). A duplex PCR will be designed by combining these two test and optimising the test circumstances (primers, probes, reagent concentrations, temperature regimen etc.) starting from conditions used for the individual PCR's.

Timetable

Week	Activities
50	Practicing with PCR and ELISA at Immunology Laboratory UU
2	Arrival/ introduction to OVI and UP
3	Practice basics of PCR and DNA extraction/ Sample inventory
4	Single PCR <i>M.bovis</i>
5	Single PCR <i>B.abortus</i>
6	Duplex PCR optimization using spiked samples/ DNA extraction from tonsils
7	DNA extraction from buffalo tonsils: frozen and fresh samples
8	DNA extraction from buffalo tonsils: frozen and fresh samples
9	Duplex PCR buffalo
10	Duplex PCR buffalo
11	Duplex PCR buffalo
12	Duplex PCR other species/ Reporting results
13	Duplex PCR other species/ Reporting results
14	Reporting results
15	Reporting results/ Presentation of findings

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