

## Special Bacterial Pathogens Reference Unit

### BACKGROUND

The Unit provides unique diagnostic and reference services for certain dangerous bacterial zoonotic diseases, namely plague, anthrax and botulism, and for this purpose operates a high security biosafety level 3 (BSL3) laboratory. The Unit is a WHO network laboratory for plague and anthrax and the Unit's expertise in laboratory diagnosis of plague and anthrax is acknowledged by the WHO. Additionally, several other zoonotic infections are under investigation, including leptospirosis, bartonellosis, brucellosis and tularemia.

### ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

#### ACCREDITATION AND TRAINING

The Unit was re-accredited by SANAS in October 2008. During the year staff attended various technical training courses: SANAS accreditation, dangerous goods shipment, first aid, fire training and software. Microbiology and clinical pathology registrars were given introductory training in BSL3 laboratory principles and the activities of the Unit.

#### RESEARCH

##### Molecular epidemiology of plague in southern Africa

The early 1990s saw a world-wide resurgence of human plague, culminating in the World Health Organization's inclusion of plague on its list of re-emerging diseases. Africa, particularly the sub-Saharan region, is the continent most affected by plague. With the precise molecular techniques available today it is possible to study the organism in depth, its spread from one geographic area to another, and mechanisms by which it adapts to varying ecological niches. The molecular typing methods used include rRNA gene restriction patterns (i.e. ribotyping) and pulsed field gel electrophoresis. Results obtained thus far show that 90% of the strains from the SBPRU culture collection appeared to be ribotype B (as expected in southern Africa). Pulsed field gel electrophoresis is currently being done on the strains that have been ribotyped. (Researcher: LArntzen)

##### Leptospirosis pilot surveillance study

Leptospirosis is a well-known cause of febrile illness in

many areas of the world, but its importance in South Africa is not known. It has been recognized as a cause of sporadic disease and outbreaks elsewhere in Africa. Recent interest has been stimulated by surveys of humans and rodents in southern Africa, which showed serological evidence of previous exposure, and a small number of human cases have been recently identified locally. There is a lack of awareness of the disease amongst clinicians, and the absence of sensitive and specific laboratory tests suitable for use in routine diagnostic laboratories; in contrast, in the veterinary field, animal infections have been historically well-catered for by the reference microagglutination test (MAT). We are applying two methods of detection: a screening test (ELISA) that demonstrates the prevalence of antibodies in the patients' serum, and PCR, targeting the ribosomal gene sequences specific for pathogenic leptospires. To date small numbers of samples have been tested, but we hope to improve awareness of this infection amongst clinicians and generate interest in sending clinically-relevant specimens. (Researchers: LArntzen, J Rossouw)

##### Bartonella species in human and animal populations in Gauteng Province

*Bartonella* is a genus of emerging and reemerging pathogens responsible for various clinical and sub-clinical manifestations. *Bartonella* spp. infections have been unknown and under-studied in South Africa. The deficit in the knowledge of the burden and epidemiology of *Bartonella*-related infections is the rationale for the *Bartonella* study. The specific objectives were to determine the prevalence of *Bartonella* infections in HIV-positive patients, when compared a healthy sample group; to investigate cats, dogs, and commensal and wild rodents for carriage of bartonellae; to determine the involvement of fleas from various animals (cats, dogs, rodents) as hosts and possible vectors of bartonellae; and to identify *Bartonella* species found in humans and animals. This project encountered various limitations, such as the dependence on outside collaborators for the collection of animal blood, and difficulties in recruiting and sampling healthy volunteers. The project has yielded some interesting results, which include the isolation of at least three previously undescribed strains of rodent-borne *Bartonella* spp., and information about the seroprevalence of *Bartonella* infections in humans in Gauteng Province. Molecular methods are currently being optimised for detection of *Bartonella* DNA in humans and animal blood. (MSc (Med) student: AN Trataris)

### Tularemia pilot study

*Francisella tularensis* is a small Gram-negative coccobacillus. Tularemia is primarily a disease of wild animals, perpetuated in nature by ectoparasites, environmental contamination, predation, and chronic carriage. Human infection is incidental and is usually the result of interaction with wild animals and their environs. This organism has not been reported in South Africa. There are two main biovars, type A (*F. tularensis tularensis*) and type B (*F. tularensis palearctica*), with some additional local subgroups currently proposed. Thus far, type A is only found on the North American continent. Type B is widespread in most parts of the temperate zone in the northern hemisphere, including North America. Only a few poorly documented cases have been reported from the southern hemisphere, with no mention of biovars. This project involves using immunofluorescence and haemagglutination tests to detect antibodies in rodent and human sera. (Researcher: LArntzen)

### *Clostridium botulinum* prevalence in Zoo Lake, Johannesburg

*Clostridium botulinum* is a Gram-positive, spore-forming, toxin-producing anaerobic bacterium that is a serious disease risk for humans and animals. Sources of botulism include the environment (i.e. water and soil) and contaminated food, especially damaged canned food or incorrectly-preserved food. The neurotoxin causes acute flaccid paralysis and ultimately death in many cases, as it is one of the most potent substances known.

An investigation was carried out to determine whether *C. botulinum* is present in the water and soil at Zoo Lake, Johannesburg. There have been several outbreaks of waterfowl deaths reported in the area, allegedly due to botulism. Molecular and culture techniques were used for detection of the bacteria, and molecular techniques were used to detect the toxin-coding genes.

No *C. botulinum* was isolated; however, this could be a result of climate and seasonality. Further studies are planned for 2009. (BSc Honours student: AN Ruis)

### Brucellosis pilot study

Brucellosis is a zoonotic disease of cattle and other domestic stock. In cattle it is caused almost exclusively by *Brucella abortus*; however, *B. suis* or *B. melitensis* are occasionally implicated. Infection spreads rapidly and causes abortions in unvaccinated cattle. After exposure, cattle may shed the organism in milk for variable lengths of time in most cattle, for life. Natural transmission in cattle occurs by ingestion of organisms, which are present in large numbers in aborted fetuses, fetal membranes and uterine discharges. Cattle may also ingest contaminated feed and water. Brucellosis is the cause of febrile illness in many areas of the world, but its importance as a human disease in South Africa is not known. It has been recognized as a cause of sporadic disease and outbreaks elsewhere in Africa. Humans usually contract brucellosis from

unpasteurized milk or soft cheese made from it. Diagnosis is based on bacteriology or serology, but follow-up serology is not often sent and patients are usually treated. A pilot study will look at people at occupational risk, for example, abattoir workers, vets and farm workers.

### QUALITY ASSESSMENT ACTIVITIES

A plague EQA programme is produced for WHO-AFRO as part of a larger WHO-NICD collaboration and this is sent out three times a year to 18 plague-endemic countries in Africa, as well as India and Madagascar.

### PLAGUE SURVEILLANCE

Plague in South Africa is apparently in a quiescent phase, but experience in other countries (India, Algeria) has shown that plague activity can resume unexpectedly after decades of quiescence, and cause major disruption to medical and social/economic systems and, especially, to tourism. Plague surveillance is in the process of being re-launched by the National Department of Health. This will start in KwaZulu-Natal Province and the other historically plague-endemic provinces will follow. All plague surveillance samples (rodents, fleas, dog sera) will be sent to the SBPRU for processing.

### COLLABORATIONS

University of Hohenheim, Stuttgart, Germany (Dr W Beyers): molecular epidemiology of southern African strains of *B. anthracis*.

Protechnik Laboratory, Pretoria (Dr T Woods): rapid dipstick detection methods for plague, anthrax and botulism.

Pretoria University (Dr Henriette van Heerden): anthrax and brucellosis.

ARC and OVI Pretoria (Dr Andrew Potts): leptospirosis culture, identification and molecular testing of different serovars.

### CAPACITY BUILDING

Registered students	
MSc (MED)	AN Trataris
MSc (MED) (commencing)	AN Ruis

Graduated	
BSc (Hons)	AN Ruis
Dip Biomed tech	N Bakana