



## ADVISORY COMMITTEE ON DANGEROUS PATHOGENS

### CATEGORISATION OF STERNE STRAIN OF *BACILLUS ANTHRACIS*

#### Issue

1. The ACDP's 'Approved List of biological agents' categorises *Bacillus anthracis* as a Hazard Group (HG) 3 pathogen. Advice is sought from the members on whether it is appropriate to work with the 'Sterne strain', an attenuated non-capsulated live variant, of *B. anthracis* at containment level 2.

#### Background

2. Where the risks of infection for a particular biological agent with an approved classification are different to that expected, Control of Substances Hazardous to Health (COSHH) Regulations permit employers to reassess and reclassify the agent in consultation with the Health and Safety Executive (HSE). The reclassification needs to take account of the agent's nature and properties and should assign the agent, based on the risk of infection, to one of the appropriate groups in Schedule 3 of COSHH.
3. ACDP have categorised *B. anthracis* as a HG3 pathogen in the 'Approved List of biological agents'. The categorisation is based upon the bacteria being pathogenic to both humans and animals, the properties of the bacteria including its capacity to cause serious harm, its ability to spread into the community and the severity of infection particularly following an inhalation route of exposure.

#### The Bacteria & Infection

4. *Bacillus anthracis*, a spore-forming, gram-positive, rod-shaped, pathogenic bacterium found throughout the world, is the causative agent of anthrax in mammals. Outbreaks occur in both wild animals and domestic livestock usually contracting the disease through ingestion of soil-borne anthrax spores and die acutely. Exposure to spores may result from entry through skin abrasions (or percutaneous injury), ingestion, or inhalation. The handling of animals or animal products such as hides (e.g. drum making), horse hair plaster and wool can result in human exposure. Following exposure, anthrax spores enter the vegetative stage in which they grow and divide; and multiply in, the lymph nodes of susceptible animals/humans. When vegetative cells escape from the animal body and are exposed to oxygen, they form spores. The spores are highly resistant to heat, cold, chemical disinfectants, and drying. Spores are reported to have survived for years in the environment.

## Virulence

5. Prototypical virulent strains of *B. anthracis* synthesise three anthrax toxins and a poly-D-glutamic acid capsule, known to be involved in virulence. The structural genes for the toxin proteins are located on plasmid pXO1 and an operon encoding biosynthetic enzymes for the capsule are found on plasmid pXO2. The capsule confers anti-phagocytic properties on the bacteria and facilitates dissemination from the site of exposure. The three toxin proteins PA (protective antigen), LF (lethal factor) and EF (edema factor) are encoded by non-contiguous genes *pagA*, *lef* and *cya* respectively and regulated by a global regulator (*AtxA*), all of which are located on pXO1. The PA, is responsible for binding and toxin entry into cells, whereupon it is cleaved and forms a toxin complex with EF or LF. The PA-LF or PA-EF complex enters cells by endocytosis. The change in pH in the endosome results in conformational change in the complex, pore formation in the cell membrane and delivery of LF or EF into the cell cytosol (Heninger et al 2006).
6. *In vivo* studies, using toxigenic or capsular mutants have demonstrated that the capsule is essential for bacterial dissemination from the site of exposure and virulence in the murine model for inhalation anthrax (Drysdale et al 2005) and that the toxin proteins are not required for lethality but do effect the course of infection (Heninger et al 2006). Taken together these studies indicate that when present, the capsule is the prevailing virulence factor.

## Sterne Strain

7. Annual vaccination of livestock in endemic anthrax areas is recommended. The live attenuated Sterne-strain vaccine is widely used for the prevention of anthrax in animals and consists of an adjuvant combined spore suspension, produced in the United States and licensed for use in livestock (cattle, sheep, horses, goats, and swine). Sterne developed this attenuated live animal vaccine in 1935 and its derivatives account for almost all vaccines used in the world today. Although an effective vaccine in animals, the Sterne vaccine does retain some virulence, particularly in goats, llamas and occasionally other animals, which may die following vaccination (Turnbull, 1991).
8. The Sterne strain lacks the plasmid pXO2, but retains plasmid pXO1 hence the bacteria produces toxins but no capsule. The bacteria are therefore susceptible to phagocytosis and opsonisation, with expected rapid clearance in healthy individuals. Attenuated vaccine strains (STI-1 and No.3) equivalent to Sterne (Merabishvili et al 2006), which lack pXO2 but retain pXO1 have been used for large scale vaccination of humans in the former Soviet Union, via a variety of exposure routes with few reported adverse side effects. Although licensed for use in at-risk occupations in the former Soviet Union, there are numerous contraindications that preclude use of attenuated spore vaccine in humans and in the Western world, the live spore vaccine is considered unsuitable for human use (Shlyakhov & Rubinstein, 1994, Turnbull, 1991).
9. Although lacking the pXO2 plasmid, there may be a theoretical risk that the Sterne strain could acquire the plasmid from wild type bacteria or a capsulated non-toxigenic strain (e.g. Pasteur strain pXO1-, pXO2+) as both plasmids have been shown to be mobilizable via horizontal DNA-transfer (e.g. mediated by

conjugative plasmids, which are often present in related species of *Bacillus*). However, in the absence of this exogenous DNA, reversion of the Sterne strain to full virulence is highly unlikely.

### International Perspective

10. In the USA, the publication '*Biosafety in Microbiological and Biomedical Laboratories*' recommends biosafety level 2 for laboratory work with bacteria such as Sterne strain. In addition, the Select Agent list of potential bioterrorist agents, excludes attenuated strains of *B. anthracis* such as Sterne based upon reported attenuation of  $10^5$  to  $10^7$  fold less virulence than isogenic strains with both virulence plasmids and their use to vaccinate both humans and animals without adverse effects on public health and safety (Shlyakhov & Rubenstein 1994, Hambleton et al, 1984).

### Action

- Members are asked to consider the available evidence and provide advice on whether the Sterne strain is sufficiently attenuated particularly via the airborne route of exposure, to warrant working with this strain at containment level 2?
- Are there circumstances under which re-categorisation is not appropriate (e.g. large scale production)?

### Secretariat

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### References

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