An Introduction to the Principles and Practices of Biosafety

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University of Hong Kong, Biological Safety Officer

ARRANGEMENTS
Slides will shortly be on the Safety Office website under the biosafety pages and include a large number of sources of information etc.
Break - 5 mins about half way through.

TOPICS
Introduction
Laboratory Acquired Infections.
Aerosols/Hierarchy of Control.
Biosafety Cabinets.
Classification of organisms according to hazard/risk.
Biosafety levels.
Hong Kong Law.
Clinical waste.
HKU arrangements / Risk assessment

AIMS
On completion participants should have a general understanding of the principles of Biosafety and be able to find further detailed information on specific topics.

Dr Mike Mackett, DipOSH, CMIOSH
University of Hong Kong,
Assistant Director of Safety
Biological Safety Officer 2005 - present

Experience:--
Research in Molecular Virology:--
PhD London, 1981; NIH USA 1983;
Manchester UK 1983-2000
UK government Specialist Inspector in Biotechnology, Liverpool, 2000-2005

What do we mean by Biosafety or Biosecurity? - a few definitions

Biological safety - Biosafety
Aim is to reduce or eliminate accidental exposure to, or release of, infectious agents (includes Bacteria, Fungi, Viruses, Parasites and cell culture)

Biosecurity
Aim is to protect against theft or diversion of hazardous agents.

Anthrax incident/ select agents list in US (late 90's - new list 2005)
Anti-Terrorism, Crime and Security Act (2001,2007) UK (NaCTSOs)
HK import export controls on specified chemical and biological agents
Effective Biosafety complements Biosecurity

Biosafety and Infection control inform Biosecurity

BIORISK

BIOSAFETY

Access, Facility Design, Sample Storage etc.

Access, Facility Design, Sample Storage etc.

BIOSECURITY

BIORISK encompasses both biosafety and biosecurity

HK - East Asian Games 2009 exercise to prepare for chemical, biological or nuclear attack

HK Emergency preparedness exercise - for disease outbreak - 25/10/10

"Laboratory Acquired Infections - LAI"

Consequences of LAI

1. Morbidity and occasional mortality - historically >5000 cases with >200 deaths
2. Personal Costs - reputation etc
3. Financial Costs - to community and University
4. Increased State supervision - "Legislation etc"
5. Significant inconvenience.

Laboratory Acquired Infections (LAI)

Definition:

An infection that is acquired through laboratory or laboratory related activities.

The infection can be:

- Symptomatic or Asymptomatic
- Human or Animal - Zoonotic
- Viral, Bacterial, Parasitic or Fungal
- from Research, Teaching, Diagnostic or Production

Some LAI reports include secondary infections to family members etc.

First reports of LAI's

<table>
<thead>
<tr>
<th>Disease</th>
<th>Year</th>
<th>Associated Incident</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>Cholera</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>Diphtheria</td>
<td>1900</td>
<td></td>
</tr>
<tr>
<td>Tetanus (J)</td>
<td>1893</td>
<td>Accidental self-inoculation *1</td>
</tr>
<tr>
<td>Typhoid (J)</td>
<td>1885, 1886, 1893</td>
<td>Mouth pipetting *2</td>
</tr>
</tbody>
</table>

*1 Nicolas (1802) "Sur un cas de Tetanos chez un homme par inoculation accidentelle des produits solubles des Bacilli Nicolaïer." Compt. Rend. der. Séances de la Société de Biologie 5, 544-547

*2 Kisskalt (1915), "Laboratory Infections with Typhoid Bacilli." Zeitschrift fur Hygiene und Infektionskrankheiten, 80 pp 145-162

Symptomatic LAIs by Time Period and Agent Category

What can we do with the data?

Limitations:

i) A literature review is not an epidemiological survey
ii) Data mostly limited to English language publications
(Sevilla-Reyes - 2009 ABSA conference abstract did list 1,179 laboratory exposures in Spanish and Portuguese language Journals).

Also see (article in Hebrew) - A hantavirus killed an Israeli researcher: hazards while working with wild animals. Harefuah 2014; 153(8): 443-4, 499.

Are the data useful?

Case studies reinforce training and program guidance.

Brits Rush to Contain Foot-and-Mouth

Monday, Aug. 06, 2007 BY EBEN HARRELL

"The virus escaped from a research laboratory"

Officials stand amongst slaughtered cows at a farm near Guildford in a bid to contain the latest outbreak of the highly infectious foot-and-mouth disease.

Most Frequently Reported LAIs

<table>
<thead>
<tr>
<th>Infectious Agent</th>
<th>Rank Order (of cases 1930-1978)</th>
<th>Infectious Agent</th>
<th>Symptomatic Cases (1979-2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella spp.</td>
<td>426</td>
<td>M. tuberculosis</td>
<td>199</td>
</tr>
<tr>
<td>Coxiella burnetii</td>
<td>280</td>
<td>Coxiella burnetii</td>
<td>192</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>258</td>
<td>B. Tuberculosis</td>
<td>195</td>
</tr>
<tr>
<td>B. Tuberculosis</td>
<td>235</td>
<td>H. Tuberculosis</td>
<td>198</td>
</tr>
<tr>
<td>H. Tuberculosis</td>
<td>214</td>
<td>E. coli</td>
<td>143</td>
</tr>
<tr>
<td>E. coli</td>
<td>212</td>
<td>H. dermophilia</td>
<td>162</td>
</tr>
<tr>
<td>H. dermophilia</td>
<td>212</td>
<td>L. monocytogenes</td>
<td>82</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>212</td>
<td>EEE</td>
<td>146</td>
</tr>
<tr>
<td>EEE</td>
<td>146</td>
<td>Shigella spp.</td>
<td>66</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>115</td>
<td>Shigella spp.</td>
<td>64</td>
</tr>
<tr>
<td>C. parvum</td>
<td>115</td>
<td>Shigella spp.</td>
<td>64</td>
</tr>
<tr>
<td>C. parvum</td>
<td>115</td>
<td>H. influenzae</td>
<td>46</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>115</td>
<td>Pseudomonas</td>
<td>32</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>115</td>
<td>Lach. C virus</td>
<td>82</td>
</tr>
<tr>
<td>Lach. C virus</td>
<td>115</td>
<td>N. meningitidis</td>
<td>31</td>
</tr>
</tbody>
</table>

1 case from contaminated samples - Singapore
1 case from exposure to spilled material - Taiwan
4 cases from incomplete inactivation of samples - Beijing

Biosafety and SARS Incident in Singapore September 2003

Report of the Review Panel on New SARS Case and Biosafety

http://www.wpro.who.int/sars/docs/pressreleases/mr_24092003.pdf

A voluntary online survey from 2002-2004 of US diagnostic laboratories revealed at least 33% had one known laboratory acquired infection

41 bacterial LAI were reported
Shigella (15)
Brucella (7)
Salmonella spp (6)
Staphylococcus aureus (6) with 5 of them being methicillin resistant (i.e. MASA)
N. meningitidis (4)
Ecoli O157:H7 (2)
Clostridium Difficile (1)


<table>
<thead>
<tr>
<th>Accident</th>
<th>No. (%) of infections reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splashes, sprays and spills</td>
<td>188 (26.7)</td>
</tr>
<tr>
<td>Needlesticks</td>
<td>177 (25.2)</td>
</tr>
<tr>
<td>Sharp objects</td>
<td>112 (15.9)</td>
</tr>
<tr>
<td>Animal or ectoparasite bite/scratch</td>
<td>95 (13.5)</td>
</tr>
<tr>
<td>Mouth pipetting</td>
<td>92 (13.1)</td>
</tr>
<tr>
<td>Other, unknown</td>
<td>39 (5.5)</td>
</tr>
<tr>
<td>Total</td>
<td>703</td>
</tr>
</tbody>
</table>

Connected to an accident

18%
82% unknown

Labs in which Infections Occur
Adapted from Pike, 1974; Harding and Byers, 2006

<table>
<thead>
<tr>
<th>Type of Facility</th>
<th>1930-1975</th>
<th>1975-2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research</td>
<td>59%</td>
<td>50%</td>
</tr>
<tr>
<td>Clinical/Diagnostic</td>
<td>17%</td>
<td>45%</td>
</tr>
<tr>
<td>Teaching</td>
<td>3%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Other or unspecified</td>
<td>21%</td>
<td>4%</td>
</tr>
</tbody>
</table>

Difficulties with figures - Are you sure it's an LAI?

LAI's are under-reported

Lab workers fail to report - Various reasons, mild cases, fear of sanction etc. e.g. Taiwan SARS case.
No legal requirement (except in some countries/circumstances).
Community vs Laboratory acquisition for some agents e.g. M.tb, Influenza and HIV
Asymptomatic infection
Long incubation period for some agents e.g. Hep B/C and Mtb
Specific accidents present in only 20% of LAI
Other complicating factors e.g. BCG and Mantoux test for M.tb

Discuss scenario

Case Study illustrates:

- How laboratory infections can occur
  1. In this case, probably by direct contact from droplets
  2. Should think of agent factors such as infective dose, transmissibility, etc.
- How a risk assessment should be done, taking into account the hazardous factors:
  1. The agent (how transmitted)
  2. Steps taken in the protocol
  3. Human behavior (touching the face)
- Preventive measures
  1. Decontamination
  2. Proper personal protective equipment (PPE)
  3. Biological safety cabinet (BSC) for manipulation of the sample
  4. Correct use of the BSC
- Administrative procedures for reporting laboratory-associated infections (LAIs)
  1. Reporting procedure
  2. Medical care
  3. Follow up
  4. Review of the case
  5. Modification of the protocol
  6. Re-training
  7. Laboratory audit

General guidelines for spill procedures

<table>
<thead>
<tr>
<th>Location</th>
<th>Volume/ Infection</th>
<th>BSL1/2</th>
<th>BSL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside BSC</td>
<td>&lt;5ml and or &lt;10^9/ml</td>
<td>Clean yourself</td>
<td>Decontaminate immediately</td>
</tr>
<tr>
<td></td>
<td>&gt;5ml and or &gt;10^9/ml</td>
<td>Consider stopping work</td>
<td>Don't let dry, Leave cabinet on</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outside BSC</td>
<td>&lt;5ml and or &lt;10^9/ml</td>
<td>Simple clean up</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;5ml and or &gt;10^9/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrifuge</td>
<td>any</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Noteworthy LAI's**

http://environmentalhealthandsafetyoffice.dal.ca/files/LAI's.pdf

**Neisseria meningitidis** (31 symptomatic LAIs)
- high case fatality rate (~50%)
- cases associated with organism i.d. and plate reading, subculturing, preparing suspensions
- CDC report: in 15 of 16 cases work not performed in BSC

**Salmonella spp.** (64 symptomatic LAIs)
- many cases associated with proficiency panels, including one case (fatality) in the family of a laboratory worker
- obvious breakdown in safe lab techniques
- CDC report: child whose mother was a lab worker developed typhoid; mother ate her lunch in the lab after working with S. typhi cultures, then brought her half eaten sandwich home for her son to finish

**Multiple Salmonella typhimurium outbreaks linked to clinical and teaching microbiology laboratory exposure.**

1st Occurred August 2010 – June 2011 see:-
http://www.cdc.gov/salmonella/typhimurium-laboratory/011712/index.html
- sickened 109 people in 38 states
3rd outbreak details:-
https://www.cdc.gov/salmonella/typhimurium-07-17/index.html

Illnesses involve a commercially available Salmonella enterica serotype Typhimurium strain used in laboratories
- strain, commonly used as a control in testing, "isn't known to be unusually pathogenic.

Health officials believe students or lab employees may have carried the bacteria to their homes on contaminated lab coats, pens, notebooks, or other items.
- Several of the patients are children who live in households with a person who studies or works in a microbiology lab.
  - Ages range from less than 1 year to 91 years, median age: 24.
  - Sixty-three percent of the patients are female;
  - 12% of the patients hospitalized, 1 death reported

**Laboratory associated exposure to orthopoxviruses reported to CDC (2005-8).**

<table>
<thead>
<tr>
<th>Year</th>
<th>State</th>
<th>Virus (strain, if known)</th>
<th>Nature of accident</th>
<th>Result in infection?</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>CA</td>
<td>Vaccinia</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2005</td>
<td>FL</td>
<td>Vaccinia (rabiespox)</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2005</td>
<td>CT</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2005</td>
<td>PA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>Yes</td>
</tr>
<tr>
<td>2006</td>
<td>CT</td>
<td>Vaccinia</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2006</td>
<td>CA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2006</td>
<td>CT</td>
<td>Vaccinia</td>
<td>Eye splash</td>
<td>No</td>
</tr>
<tr>
<td>2006</td>
<td>MA</td>
<td>Vaccinia</td>
<td>Needlestick</td>
<td>Yes</td>
</tr>
<tr>
<td>2006</td>
<td>MA</td>
<td>Monkeypox</td>
<td>Needlestick</td>
<td>No</td>
</tr>
<tr>
<td>2006</td>
<td>MA</td>
<td>Vaccinia</td>
<td>Animal care facility</td>
<td>No</td>
</tr>
<tr>
<td>2006</td>
<td>MA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>Yes</td>
</tr>
<tr>
<td>2007</td>
<td>MA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2007</td>
<td>MA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2007</td>
<td>MA</td>
<td>Vaccinia (recombinant WR)</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
<tr>
<td>2007</td>
<td>MA</td>
<td>Vaccinia</td>
<td>Needlestick</td>
<td>Yes</td>
</tr>
<tr>
<td>2008</td>
<td>MA</td>
<td>Vaccinia</td>
<td>Needlestick</td>
<td>No</td>
</tr>
<tr>
<td>2009</td>
<td>MA</td>
<td>Vaccinia</td>
<td>Needlestick</td>
<td>No</td>
</tr>
<tr>
<td>2010</td>
<td>MA</td>
<td>Vaccinia</td>
<td>Needlestick</td>
<td>No</td>
</tr>
<tr>
<td>2010</td>
<td>MA</td>
<td>Vaccinia</td>
<td>Needlestick</td>
<td>Yes (hospitalization)</td>
</tr>
</tbody>
</table>


**Vaccinia lesions and EM of virus isolates**

Senior scientist wore no gloves even though he was aware of a cut on his knuckle.
### Laboratory Acquired Infections with Biological Select Agents or Toxins (USA)

Data from Applied Biosafety (2012) 17(4), 171-180. LAIs occur even with the most regulated set of agents.

<table>
<thead>
<tr>
<th>Year</th>
<th>Agent</th>
<th>Cases</th>
<th>Entity Type</th>
<th>Lab Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Brucella militensis</td>
<td>1</td>
<td>Registered</td>
<td>BSL2</td>
</tr>
<tr>
<td>2004</td>
<td>Coccidiosis sp.</td>
<td>1</td>
<td>Registered</td>
<td>BSL3</td>
</tr>
<tr>
<td>2004</td>
<td>Francisella tularensis</td>
<td>3</td>
<td>Registered</td>
<td>BSL2</td>
</tr>
<tr>
<td>2007</td>
<td>Brucella militensis</td>
<td>1</td>
<td>Registered</td>
<td>BSL3</td>
</tr>
<tr>
<td>2007</td>
<td>Francisella tularensis</td>
<td>1</td>
<td>Exempt</td>
<td>BSL2</td>
</tr>
<tr>
<td>2009</td>
<td>Brucella militensis</td>
<td>1</td>
<td>Exempt</td>
<td>BSL3</td>
</tr>
<tr>
<td>2010</td>
<td>Brucella suis</td>
<td>1</td>
<td>Exempt</td>
<td>BSL2</td>
</tr>
</tbody>
</table>

### Routes of Exposure and Lab Work

**Injestion:** eating in the lab, mouth pipetting, transfer of agent to the mouth by contaminated fingers or articles

**Inoculation:** needlesticks, cuts, animal bites and scratches

**Contamination of the skin and Mucous membranes:**
- Splashes – mouth, eyes, nose
- Contaminated surfaces

**Inhalation:** numerous procedures that produce aerosols

Exposure to aerosols may be the greatest biohazard facing laboratory workers (Collins)

### Risk Factors for Laboratory Acquired Infections – Slide “borrowed” from Prof. Yuen, 2004

- Immunodeficiency
- Vaccination status
- Low opinion of safety programs
- Take risks
- Work too fast
- Lack of awareness of the agent being worked
- Young (17-24) male workers
- Self non-complied change of SOP
- Lack of team spirit and openness in the laboratory
- Lack of oversight of each other (- the director is worse)
- Draconian policy leading to hiding of accidents
- Incomplete/wrong inventory of infectious samples

### Resources for LAIs


Collins (bibliography of LAIs - 1999): [http://www.boku.ac.at/iam/efb/lai.htm](http://www.boku.ac.at/iam/efb/lai.htm)
