Biosecurity and biosafety in the laboratory: Experience of the OIE Reference Laboratory for Avian Influenza

Isabella Monne (DVM, PhD)
National OIE/FAO Reference Laboratory for Newcastle Disease and Avian Influenza
Istituto Zooprofilattico Sperimentale delle Venezie,
Viale dell’Università, 10 - 35020 - Legnaro (PD)- Italy

29-30 September 2015- Tunis
The possibility that an influenza infection in humans caused by avian influenza viruses A viruses could occur following a laboratory accident is a risk to which it is crucial to be constantly alert.
Which is the first thing you have to use before handling any infectious biological agent?
Which is the first thing you have to use before handling any infectious biological agent?

If my brain were an imaginary friend
Which is the first thing you have to use before handling any infectious biological agent?

Do not follow just your instinct!!
Risk Assessment

Risk Assessment depends on a coordinated approach that enables the appropriate selection of measures to ensure reasonable and adequate laboratory security without unduly affecting the scientific work.
“The most important component of risk assessment is professional judgment. Risk assessments should be performed by the individuals most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, animal models that may be used, and the containment equipment and facilities available.”

Remember....

- Protecting the public's health
- Protecting employees / co-workers
- Protecting research at the institution
- Protecting the image of the institution
Risk Assessment

Primary factors to consider:

1. Agent hazards (Risk Group)

2. Laboratory procedure hazards and hazards associated with work practices
The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use based on these principal characteristics:

- pathogenicity
- infectious dose
- mode of transmission
- host range
- availability of effective preventive measures
- availability of effective
The WHO and the OIE has recommended an agent risk group classification for laboratory use based on these principal characteristics:

- pathogenicity
- infectious dose
- mode of transmission
- host range
- availability of effective preventive measures
- availability of effective treatment

VIRAL STRAIN-DEPENDENT
<table>
<thead>
<tr>
<th>RISK GROUP CLASSIFICATION</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WHO, 2004</strong> <em>(FOR LABORATORY USE ONLY)</em></td>
<td></td>
</tr>
<tr>
<td><strong>Risk Group 1</strong> <em>(no or low individual and community risk)</em></td>
<td>A microorganism that is unlikely to cause human or animal disease.</td>
</tr>
<tr>
<td><strong>Risk Group 2</strong> <em>(moderate individual risk, low community risk)</em></td>
<td>A pathogen that <em>can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers</em>, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.</td>
</tr>
<tr>
<td><strong>Risk Group 3</strong> <em>(high individual risk, low community risk)</em></td>
<td>A pathogen that <em>usually causes serious human or animal disease but does not ordinarily spread</em> from one infected individual to another. Effective treatment and preventive measures are available.</td>
</tr>
<tr>
<td><strong>Risk Group 4</strong> <em>(high individual and community risk)</em></td>
<td>A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.</td>
</tr>
<tr>
<td>Risk Group 1</td>
<td>A microorganism that is unlikely to cause human or animal disease.</td>
</tr>
<tr>
<td>Risk Group 2</td>
<td>A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.</td>
</tr>
<tr>
<td>Risk Group 3</td>
<td>A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.</td>
</tr>
<tr>
<td>Risk Group 4</td>
<td>A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.</td>
</tr>
</tbody>
</table>
## RISK GROUP CLASSIFICATION

**WHO, 2004**  
*(FOR LABORATORY USE ONLY)*

<table>
<thead>
<tr>
<th>Risk Group 1</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(no or low individual and community risk)</td>
<td>A microorganism that is unlikely to cause human or animal disease.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk Group 2</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(moderate individual risk, low community risk)</td>
<td>A pathogen that <em>can cause human or animal disease</em> but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk Group 3</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(high individual risk, low community risk)</td>
<td>A pathogen that <em>usually causes serious human or animal disease</em> but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk Group 4</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(high individual and community risk)</td>
<td>A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.</td>
</tr>
</tbody>
</table>

Avian influenza viruses are classed at a minimum in Risk Group 2 for human and animal infection (OIE, Terrestrial Manual 2015)

Higher level containment being indicated for H5/H7 LPAI and HPAI viruses (OIE, Terrestrial Manual 2015)
Risk Assessment

Primary factors to consider:

1. Agent hazards (Risk Group)

2. Laboratory procedure hazards and hazards associated with work practices
Hazardous Characteristics of Laboratory Procedures

Routes of transmission in the laboratory for avian influenza virus:

1. Direct skin, eye or mucosal membrane exposure
2. Parenteral inoculation (syringe needle, other contaminated sharp or bites from infected animals)
3. Ingestion (e.g. hand to mouth exposure)
4. Inhalation of infectious aerosols

The first three routes of laboratory transmission are easy to detect (20% of all reported Laboratory-Associate Infections LAIs)
Hazardous Characteristics of Laboratory Procedures

Routes of transmission in the laboratory for avian influenza virus:

1. Direct skin, eye or mucosal membrane exposure
2. Parenteral inoculation (syringe needle, other contaminated sharp or bites from infected animals)
3. Ingestion (e.g. hand to mouth exposure)
4. Inhalation of infectious aerosols

The first three routes of laboratory transmission are easy to detect (20% of all reported Laboratory-Associate Infections LAIs)
Biosafety Levels (BSLs)

Four Biosafety Levels (BSL1 to 4) are designated in ascending order, by degree of protection provided to personnel, the environment and the community.
Biosafety levels (BSLs)

BSLs consist of a combination of:

- **laboratory practices and techniques** (the most important element of containment is strict adherence to standard microbiological practices and techniques)
- **safety equipment** (primary barriers and personal protective equipment-PPE)
- **laboratory facilities design and construction** (secondary barriers)
Laboratory safety measures for Avian Influenza
<table>
<thead>
<tr>
<th>RISK GROUP</th>
<th>BIOSAFETY LEVEL</th>
<th>LABORATORY TYPE</th>
<th>LABORATORY PRACTICES</th>
<th>SAFETY EQUIPMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basic - BSL1</td>
<td>Basic teaching Research</td>
<td>Good microbiological techniques (GMT)</td>
<td>None, open bench work</td>
</tr>
<tr>
<td>2</td>
<td>Basic - BSL2</td>
<td>Primary health services</td>
<td>GMT, biohazard sign, limited access</td>
<td>Open bench plus BSC (Biological Safety Cabinet) for potential aerosols, autoclave available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diagnostic services</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Research</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Containment - BSL3</td>
<td>Special diagnostic services</td>
<td>As level 2 plus special clothing, controlled access, directional airflow, decontamination of all waste</td>
<td>BSC and/or other primary devices for all activities, environmental and functional isolation, autoclave on site, exhausted air not recirculated</td>
</tr>
<tr>
<td>2 (high volumes /high conc.)</td>
<td>Containment - BSL3</td>
<td>Special diagnostic services</td>
<td>As level 2 plus special clothing, controlled access, directional airflow, decontamination of all waste</td>
<td>BSC and/or other primary devices for all activities, environmental and functional isolation, autoclave on site, exhausted air not recirculated</td>
</tr>
<tr>
<td>4</td>
<td>Maximum containment - BSL4</td>
<td>Dangerous pathogen unit</td>
<td>As level 3 plus airlock entry, Clothing change before entering, shower on exit, special waste disposal (all material decont.)</td>
<td>Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double ended autoclave (through the wall), filtered air, environmental and functional isolation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RISK GROUP</td>
<td>BIOSAFETY LEVEL</td>
<td>LABORATORY TYPE</td>
<td>LABORATORY PRACTICES</td>
<td>SAFETY EQUIPMENT</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>----------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>1</td>
<td>Basic - BSL1</td>
<td>Basic teaching Research</td>
<td>Good microbiological techniques (GMT)</td>
<td>None, open bench work</td>
</tr>
<tr>
<td>2</td>
<td>Basic - BSL2</td>
<td>Primary health services Diagnostic services Research</td>
<td>GMT, biohazard sign, limited access</td>
<td>Open bench plus BSC (Biological Safety Cabinet) for potential aerosols, autoclave available</td>
</tr>
<tr>
<td>3</td>
<td>Containment - BSL3</td>
<td>Special diagnostic services Research</td>
<td>As level 2 plus special clothing, controlled access, directional airflow, decontamination of all waste</td>
<td>BSC and/or other primary devices for all activities, environmental and functional isolation, autoclave on site, exhausted air not recirculated</td>
</tr>
</tbody>
</table>

The orthomyxoviruses are risk group 2 agents. However, the measures required may vary among the subtypes, with higher level containment (e.g. Risk group 3 or 4) being indicated for H5/H7 LPAI and HPAI viruses (Terrestrial manual, OIE).

The primary laboratory hazard is inhalation of virus from aerosols generated by manipulating virus-infected samples. In addition, laboratory infection can result from direct inoculation of mucus membranes through virus-contaminated gloves.
Avian Influenza outbreak and problems encountered by a diagnostic laboratory

- Alert and delivery of samples on unusual time (extra working hours, week-ends, holidays)
- Sudden increase in sample testing (up to several hundreds/week)
- Pressure for faster turn-around-time (TAT)

In combination with

- High quality test performances
- Cost effectiveness
H7N7 HPAI – Italy 2013 Index case (Laying hen- Industrial farm)
• **13 August** – afternoon: alert (on 12 August a peak of 1188 dead birds out of a total of 23,700 hens was registered)

**H7N7 HPAI – Italy 2013 Index case (Laying hen- Industrial farm)**
Assess the risk and identify the BSL

1. Risk group???

2. Biosafety level???
Strutture BSL3 dell’Istituto
Biosafety actions (BSL3):

1. Supervisor should immediately provide guidelines for safe packaging of the potentially infectious materials (basic triple packaging system)

2. Alert the BSL3 supervisor (if different from the person possessing the information related to the outbreak)

To be continued
Biosafety actions (BSL3):

3. Supervisor: decide who enter (at least two persons)

4. All persons handling the samples must be advised of the potential hazards and meet specific entry/exit requirements

5. Only laboratory personnel who received specific training in handling pathogenic and potentially lethal agents can enter the BSL3 facilities

To be continued
Laboratory staff must be fully trained in the:

- Handling of pathogens
- Use of safety equipment
- Disposal techniques
- Handling of contaminated waste
- Emergency response

Health check up for the lab staff
H7N7 HPAI – Italy 2013 Index case (Laying hen- Industrial farm)

13 August – afternoon: alert (on 12 August a peak of 1188 dead birds out of a total of 23,700 hens was registered)

14 August: tracheal and cloacal swabs sent to the Reference Laboratory (RL).

14 August – 8:30am. Sample identification and registration at the RL.
Biosafety actions (BSL3):

6. Identified and registered potentially infectious materials must be placed in a durable, leak proof container and passed in BSL3 through a pass-box.

7. BSL3 authorized personnel: restricted access to the laboratory is through two self-closing doors.

To be continued...
BSL3 equipment

1. Negative air pressure, HEPA filtration, no recirculation
2. Autoclave and BSC
3. Personal Protection Equipment (PPE): head covers, footwear, gloves and appropriate respiratory protection
4. Double door entry and body shower
5. Biohazard sign
6. Emergency tools
7. Storage facilities
Biosafety actions (BSL3):

8. BSL3 personnel apply following standard and special safety practices:
- Wear protective laboratory clothing

To be continued
Principal aspects for PPE management:

- Appropriate selection and training on proper use
- Cleaning, maintenance, repair
- Storage
- Individual needs
And don’t forget laboratory shoes!

No sandals or open-toed shoes in the BSL-2 (or any) laboratory. Appropriate footwear.
Personal Protective Equipment (PPE)

- Disposable tyvek overall with hood
- Resistant shoe-covers
- Rubber boots
- Disposable gloves (2 pairs)
- Protective glasses
- Respiratory masks with aspiration valve
- Full face mask with P3 or HEPA filter
Biosafety actions (BSL3):

9. Potentially infectious materials collected from the passbox must be handled within a BSC II or III. The trained staff proceed with extraction of RNA from the HPAI suspected samples.
Under the Cabinet

Do not place too many things under the Biosafety Cabinet!
Under the Cabinet

Work at least 10 cm inside the Biosafety Cabinet
A good organization of both working material and equipment under the Biosafety Cabinet can increase the safety for the operator and reduce the risk of contamination.
A good organization of both working material and equipment under the Biosafety Cabinet can increase the safety for the operator and reduce the risk of contamination.
Biosafety actions (BSL3):

10. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

11. Tubes containing extracted RNA removed from the facility must be appropriately decontaminated and moved outside the BSL3 building using passbox.
12. Remove PPE in the following order:

1. GLOVES 1° PAIR
2. OVERALL
3. GOGGLES
4. FACE MASK
5. BOOTS
6. GLOVES 2° PAIR

Disposable PPE must be properly discarded (sealed plastic bags). Reusable or non disposal PPE should be cleaned and disinfected in suitable way.

COMPLETE HYGIENE MUST BE PERFORMED AFTER REMOVING PPE
HPAI Workflow

1. HPAI sample
2. Viral Growth (eggs or cells)
3. RNA extraction
4. PCR
5. Sequencing

BSL3

BSL2
LPAI Workflow

LPAI sample

Viral Growth (eggs or cells)

RNA extraction

PCR

Sequencing
Health considerations

Annual vaccination with influenza human vaccine is recommended to the staff that is exposed to the infection risk to avoid recombination between avian and human virus.

Possibility to use antivirus medicines depending on the risk of exposure. The doctor in charge will have to provide to the people under infection risk, instructions about the possible use of the antivirus medicines, both for preventive and therapeutic purpose.

Personnel must communicate as soon as presenting any influenza like symptoms to the doctor in charge.
Definitions

• **Biosafety**

  Containment principles, technologies and practices that are implemented to prevent *unintentional* exposure of individuals and the environment (= biocontainment) to potentially hazardous biological agents.

• **Biosecurity**

  Institutional and personal security measures designed to prevent loss, theft, misuse or *intentional* release of microorganisms, biological materials and research-related information.
STORAGE OF PATHOGENS

Storage of live pathogens requires appropriate containment and security to avoid risks due to breakage or unauthorised use of material.

Storage facilities should be appropriately labelled to indicate the nature of the pathogens (e.g. their Group) and the contact information for the person(s) responsible for them.

A complete inventory of the pathogens in storage should be kept up to date and available.
Cross-contamination of samples is a high risk factor in laboratories that use multiple microorganisms or species with different pathogenicity.
A biobank is a repository to store biological samples for their use in research. The scope of biobanks is to provide the scientific community access to scientific material and data.

Surveillance activities and scientific research at national and international levels have resulted in collection and storage of invaluable biological materials. Part of invaluable samples, collected during AI outbreaks, is now part of IZSVe Biobank.

The IZSVe Veterinary biobank assists the World Animal Health Organization in evaluating and adopting reference materials for animal disease diagnosis.

How to access
Biological materials present in the biobank can be searched online at http://biowarehouse.net. For further information: msbeato@izsvenezie.it
Conscientious and proficient laboratory staff reduces the inherent risks that attend work with hazardous agents.

Safety equipment remove or minimize the exposures to hazardous biological materials (Biological Safety Cabinets/BSCs).

Suitable design and construction of the facilities contributes to the laboratory workers’ protection.
Thank you for your attention!