

3. RUMINANTS

3.1. Introductory note

- The FMV does not have a teaching farm nor an experimental ruminant unit because it does not have sufficient space for that purpose at the ULisbon campus in Alto da Ajuda.
- It maintains a **Resident Cattle Park (PBR)** with 18 adult cows, used exclusively for teaching purposes. These animals are acquired from farms with an officially tuberculosis-free, brucellosis-free and enzootic bovine leukosis-free health status. During their stay at the FMV they never leave the PBR nor have contact with cattle from other sources. They are therefore considered low-risk animals for infection.
- The person responsible for the Resident Animals Facilities is Prof. Dr. Ana Catarina Belejo Mora Torres. The Clinical Lead responsible for the ruminants housed in the PBR is Professor Dr. George Stilwell. The Animal Health Lead is Professor Dr. Fernando Boinas.
- In turn, the Teaching Hospital does not provide medical services nor hospitalisation for cattle, but it has an **Ambulatory Service** that provides healthcare in a private network of ruminant farms with which the FMV has collaboration agreements.
- For these reasons, this chapter is subdivided into two sections: (3.2.) biosafety practices applicable to the PBR, used in practical classes of Animal Behaviour, Welfare and Handling, Animal Production, Agriculture and Environment, Pharmacology, Pharmacy and Pharmacotherapy, Medical Propaedeutics and Clinical Practice, and Reproduction and Obstetrics; (3.3.) biosafety practices applicable to the ambulatory service on private ruminant farms during practical classes of Epidemiology, Infectious Diseases and Preventive Medicine, Reproduction and Obstetrics, Farm Animal Clinical Practice and Population Medicine.

3.2. Biosecurity Practices in the Resident Cattle Park

3.2.1. General clothing and footwear

- Coveralls are the mandatory personal garment for activities in the PBR. They must be worn by everyone to mitigate the risk of transmitting pathogens to people or animals outside the PBR.
- Students store their personal belongings in the lockers and take a bag containing their blue coveralls and their white rubber boots.
- They then proceed to the Sanitary Filter adjacent to the PBR.
- Students take a pair of shoe covers, remove their shoes, put on the shoe covers and place their shoes on a shelf. Then, they put on their coveralls, put on their rubber boots and place their bag on the shelf next to their shoes. They sit on the low wall, rotate 180°, disinfect their hands with alcohol gel and proceed to the stables.
- Students, teaching staff and employees must pass through a footbath located at the entrance to the PBR, fully immersing footwear, and only then may they enter the PBR.
- Students who do not arrive properly equipped may not participate in the class. They will be warned, will have to return to the Sanitary Filter, and come back with the appropriate clothing and footwear.
- After the practical class, students follow the reverse route: they leave the PBR, pass through the footbath, and access the Sanitary Filter where they wash the boots at the boot-washing station and disinfect them. They remove the boots and the coveralls. They remove the shoe covers and place them in the rubbish bin. They sit on the low wall and rotate 180°. They store their boots and coveralls in their bag. They take their shoes from the shelf, put them on, and proceed to the General Changing Room where they remove the coveralls, store them in a personal bag, retrieve their



personal belongings from the lockers, disinfect their hands with alcohol gel, and leave the General Changing Room.

- The student's bag containing the coveralls must be kept in their personal locker or in their backpack. The coveralls must be washed whenever they are dirty.
- Coveralls must be washed at 60°C with a detergent with disinfectant action. Students wash the coveralls at home (e.g., OMO Sanitises & Cleans, effective against bacteria and viruses).
- Poster 3 summarises the instructions that students must follow whenever they have practical classes at the PBR. This poster is displayed at the entrance to the PBR

Poster 3

Instructions for students on the Standard Operating Procedures for Biosafety in force in the FMV Resident Animals Park



FMV STABLES

INSTRUCTIONS FOR STUDENTS

- 1 - Store your belongings in a locker in the General Changing Room.
- 2 - Tie back long hair.
- 3 - Take a bag with your blue coveralls and your white rubber boots.
- 4 - Lock the locker in the General Changing Room with your padlock.
- 5 - Follow the entry and exit routes of the Sanitary Filter installed in the changing room adjacent to the room with the cattle stocks/crushes.
- 6 - If you bring a water bottle, place it in a locker in the Sanitary Filter.
- 7 - In the Sanitary Filter, take a pair of shoe covers, remove your shoes, put on the shoe covers and place your shoes on a shelf. Put on your coveralls and put on your rubber boots. Place your bag on the shelf next to your shoes. Sit on the low wall, rotate 180° and proceed to the stables.
- 8 - Disinfect your hands when entering and leaving the Stables.
- 9 - When the class ends, wash your boots at the boot-washing station. Proceed to the Sanitary Filter and remove the boots and the coveralls. Remove the shoe covers and dispose of them in the rubbish bin. Sit on the low wall and rotate 180°, store your boots and your coveralls in your bag. Take your shoes from the shelf and put them on.
- 10 - Proceed to the General Changing Room, open your locker, take your belongings, store your padlock, leave the locker open and leave.
- 11 - If you have doubts, read the QR Code on the poster to consult more detailed information.

PERSONAL PROTECTIVE EQUIPMENT (PPE):

- A - Cotton coveralls, blue, personal, clean.
- B - White rubber boots, personal, clean.
- C - PPE, e.g., gloves, is provided by the FMV.

- Teachers' coveralls are delivered by a staff member from the respective department to the FMV Laundry, where they are washed with liquid bleach with antibacterial, antifungal and



antiviral properties (e.g., Peracid Asepsis) in an industrial washing machine and subsequently dried in an industrial dryer.

- Staff may wear specific sturdy and washable work footwear when not in contact with animals or their excreta. This footwear must be used exclusively at the FMV.

3.2.2. General Cleaning and Hygiene

- People entering the PBR must use the entrance accessed through the Sanitary Filter.
- Hands must be washed and disinfected with alcohol gel before and after examining each bovine.
- Disposable gloves must be worn when handling cattle.
- There is a boot washer outside the PBR, available whenever needed.
- If, during a practical class, a surface or piece of equipment becomes contaminated with organic matter, e.g., blood, it must be cleaned and disinfected immediately by the teacher(s) or students. Cleaning is the responsibility of all PBR users.
- All equipment or materials (e.g., thermometers, stethoscopes) must be disinfected before and after use.
- Equipment such as buckets must be cleaned and disinfected with 0.5% chlorhexidine after use.
- Animal transporters comply with Regulation (EC) No 1/2005 of 22 December 2004 and Decree-Law No 265/2007 of 24 July, amended by Decree-Law No 158/2008 of 8 August, which establish the obligations of transporters, organisers and keepers during animal transport, as well as Decree-Law No 142/2006 of 27 July and its amendments, which establish the mandatory cleaning and disinfection of animal transport vehicles at facilities approved by the Directorate-General for Food and Veterinary Affairs (DGAV).
- The wheels of trucks used to transport cattle are washed and disinfected outside the PBR, in front of the garage of the Farm Animal Clinical Practice and Population Medicine vehicles, using a high-pressure washer. A card is then attached specifying which disinfectant was used, to ensure the correct contact time (e.g., Aldekol DES FF, bactericidal and yeasticidal activity in 5 minutes, virucidal activity in 60 minutes).

3.2.3. Proper Cleaning

- It is of paramount importance for ruminant hygiene and to reduce potential transmission chains of parasitic and infectious agents that the park remains clean. For this purpose, faeces must be removed regularly, and the park cleaned and washed periodically.
- Caretakers clean the park and corridors daily to remove faeces and leftover feed.
- Caretakers wash the park and corridors monthly.
- If an area of the park becomes dirty outside caretakers' working hours, students and the veterinary team (teachers, clinicians and interns) must remove the faeces.
- In the case of neonates, hygiene is crucial and therefore, as soon as there is a pile of faeces or wet bedding, it must be removed by students or the veterinary team.
- Automatic drinkers must be cleaned regularly, ensuring they are functioning properly.
- Feed troughs must be cleaned every morning before feeding.
- Animals must be kept as clean as possible.
- The area around the PBR must be clean and organised, meaning no materials scattered and no personal belongings. All PBR users are expected to make an effort to tidy materials after use.



3.2.4. General Disinfection Protocol

- Since cattle remain permanently in the park due to the absence of an alternative temporary housing location, it is very important to use animal-safe and environmentally friendly disinfectants. Disinfectants based on potassium peroxymonosulfate (pentapotassium bis(peroxymonosulfate) bis(sulfate)) and oxygen-based bleach are good options because they are effective against a wide range of pathogens and are less toxic than traditional chlorine-based bleaches. Disinfectants containing quaternary ammonium compounds or based on peracetic acid are also options.
- Caretakers are trained in the proper use of these disinfectants and always follow the manufacturer's instructions for dilution and application to ensure safety.
- Examples of safe and effective disinfectants in this context:
 - Virkon S: potassium peroxymonosulfate with bactericidal, fungicidal and virucidal activity. Does not stain, does not irritate skin or eyes, is biodegradable and harmless to users, animals (even newborns) and the environment.
 - Roxycode: oxygen bleach-based, effective against bacteria, viruses and fungi.
 - Hydrolig ANIMAL: peracetic acid-based, safe for humans, animals and the environment.
 - Zoopan Kenocox: phenol-free disinfectant safe for animals and users.
- • Wear gloves that provide protection against chemical risks when handling disinfectants. Additional PPE (mask, face shield, goggles, waterproof clothing and boots) must be used whenever splashing is likely during the disinfection process.
- Wash the park, including walls, doors, feed troughs and automatic drinkers with Keno™san detergent (Cid Lines) applied as foam. Scrubbing is always necessary to remove residues and biofilms that inhibit or reduce the effectiveness of the disinfection process.
- Rinse the washed area thoroughly to remove any detergent residue (Note: some disinfectants can be inactivated by detergents or soap, therefore it is very important to rinse properly after cleaning).
- Allow the area to drain or dry as much as possible to avoid dilution of disinfectant solutions.
- Thoroughly wet floors, walls, doors, drinkers and troughs with disinfectant at the proper dilution. The disinfectant must remain in contact with surfaces for the required time.
- Remove excess disinfectant with water.
- After disinfection, remove protective clothing and wash hands

3.2.5. Footbaths and Mats

- The disinfectant solutions in the footbath installed at the entrance to the park are changed every morning by the caretakers and whenever they contain excessive dirt or are dry.
- Everyone must use the footbath. Footbaths require full immersion of boots.

3.2.6. Disinfection Protocol for Instruments and Equipment

- All instruments and equipment must be cleaned and disinfected or sterilised between use on different animals.
- Materials that are sterilised between uses undergo:
 - an initial wash in a dishwasher (long cycle);
 - sterilisation in a dry heat oven (180 °C for 3 hours).



Stethoscopes

- Personal stethoscopes (teachers and students) may be used in the PBR but must be disinfected regularly with alcohol or hand disinfectant solution (recommended: at the beginning and end of the day).
- Immediate cleaning and disinfection are required when stethoscopes are visibly dirty.

Thermometers

- Glass thermometers are prohibited at FMV to avoid mercury exposure in case of breakage.
- Digital thermometers are used instead.
- After use, they must be carefully cleaned and disinfected with wipes soaked in alcohol and/or chlorhexidine.

3.2.7. Summary List of Detergents and Disinfectants Approved for Use in the Resident Ruminant Park

- Detergents and Soaps
 - Baktolin Pure for hand washing.
 - Zoopan Kenosan for surfaces.
- Disinfectants
 - Virocid for footbaths/disinfectant mats.
 - Virkon S in case of infectious disease.
 - Keno™cox (Cid Lines) in cases of diarrhoea (e.g., calves with coccidiosis and cryptosporidiosis).

3.2.8. Food and Beverages

- Consumption of food or beverages is not permitted in the PBR.

3.2.9. Cleaning Protocols

Trucks Transporting Healthy Live Animals for Replacement / Culling

- The wheels and sides of transport trucks, whether carrying live animals or feed, are washed and disinfected with Mida Foam 193 before entering the PBR.
- The unloading area is cleaned and disinfected with Mida Foam 193 immediately after the truck leaves.
- The road in front of the PBR is swept and pressure-washed weekly by the cleaning team and disinfected with Mida Foam 193.

Resident Cattle Park

- Areas of the PBR contaminated with faeces, secretions and urine must be cleaned daily and disinfected monthly with Virkon S. Excessive use of disinfectants promotes the development of microbial resistance.
- To ensure effectiveness, disinfectants must be used on clean surfaces.
- Using the correct disinfectant dilution, as recommended by the manufacturer, provides optimal disinfectant action.
- Biofilms develop in areas with stagnant water and where disinfectant is left on dirty surfaces.
- Uneaten feed must be removed from the manger before adding new feed.



- The feed storage room must be cleaned weekly to mitigate rodent infestations.
- Rodent traps are installed in all feed storage areas (see Chapter 10 on “Pest control”).
- FMV provides halters and leads for the cattle. All tack is cleaned and disinfected by immersion in chlorhexidine solution. Tack is stored in the PBR when not in use.

3.2.9.1. Routine Cleaning of the PBR

- **Daily routines**

- Sweep the floor to remove debris.

- **Weekly routines**

- Clean and wash feed troughs.

- **Monthly routines**

- The PBR must be disinfected monthly with Virkon S.
- To ensure effectiveness, disinfectants must be used on clean surfaces.
- Using the correct disinfectant dilution, as recommended by the manufacturer, provides optimal disinfectant action.
- Biofilms develop in areas with stagnant water and where disinfectant is left on dirty surfaces.
- Brooms must be cleaned and maintained.

- **Biannual routines**

- PBR drains must be cleaned with detergent and then injected with disinfectant (Mida Foam 193). Do not fill the drain with disinfectant without cleaning it first.

3.2.10. Deceased Cattle

- In the event of the death of a resident bovine, unless otherwise indicated, a necropsy must be performed as soon as possible.
- The carcass must be transported by forklift to the Pathology Necropsy Room in a sealed, leak-proof container and stored in the cold chamber.
- If the animal dies or is euthanised outside working hours, the carcass will remain in the container covered with a heavy plastic sheet until transported to the Pathology Necropsy Room.
- After use, the heavy plastic sheet must be cleaned and disinfected.
- The forklift used to transport the carcass must be carefully cleaned and disinfected at the dock of the Pathology Necropsy Room with Mida Foam 193.

If the Pathology Service is closed (holidays and vacation periods), necropsies must be carried out by the Farm Animal Hospital team in the Necropsy Room.

3.2.11. Surveillance and Case Management

3.2.11.1. Routine Environmental Surveillance

- Routine environmental surveillance of smooth floors and hand-contact surfaces must be performed every 6 months.
- Culture results are recorded in the resident cattle database. Results are communicated to the CHB as soon as they become available

3.2.11.2. Management of Suspected or Confirmed Cases of *Salmonella* spp.

- If salmonellosis is suspected, the following measures are activated:



- The responsible veterinarian must notify the CHB as soon as possible (email sent to: biosseguranca@fmv.ulisboa.pt).
- Classes involving the use of PBR cattle are suspended until the sporadic case or outbreak is eliminated.
- The PBR may not receive new animals until the sporadic case or outbreak is eliminated.
- Isolation and treatment of the infected bovine in an isolation pen.
- Collection of individual biological samples from all cattle.
- Washing and disinfection of the PBR with Virkon S.
- After cleaning and disinfection, collection of environmental samples from PBR surfaces for bacteriological analysis.
- If cultures are positive for *Salmonella* spp., the cleaning and disinfection procedure must be repeated.
- The infected bovine(s) may only return to the PBR after three consecutive negative bacteriological results.
- The PBR may not receive new animals until the infected bovine(s) have three consecutive negative culture results.
- These results must be recorded in the resident cattle database.
- Suspected and confirmed salmonellosis cases must be reported by the responsible PBR veterinarian to DGAV (form 1728, sent to: secdspa@dgav.pt and secretariado.lvt@dgav.pt).

3.2.11.3. Management of Patients with Suspected Other Infectious Diseases

- Special measures are required in the management of patients with suspected or confirmed infection by contagious pathogens. Due to their transmission potential, the main conditions of concern include:
 - Calves with acute gastrointestinal disorders (e.g., diarrhoea) suspected of cryptosporidiosis.
 - Acute respiratory diseases of viral origin.
 - Infections with multidrug-resistant bacteria.
- Any of the following clinical signs are indicative of infectious enteric disease:
 - Fever
 - Prostration
 - Anorexia
 - Weight loss
 - Diarrhoea
 - Hypoproteinaemia.
- Any of the following clinical signs are indicative of infectious respiratory disease:
 - Fever
 - Prostration
 - Anorexia
 - Nasal discharge
 - Tachypnoea–dyspnoea
 - Cough.
- Animals showing clinical signs compatible with infectious disease will be isolated in an isolation pen:



- The CHB must be notified as soon as possible (email sent to: biosseguranca@fmv.ulisboa.pt) so that it can assess whether appropriate biocontainment practices are being implemented.
- The isolation pen must be delimited with a sanitary barrier area.
- The potential contagious risk of isolated cattle must be indicated on a sign posted on the pen.
- The number of people in contact with the patient bovine must be limited.
- When a bovine with suspected or confirmed infectious disease leaves the isolation pen, a sign stating “Do not use, special cleaning and disinfection required” must be placed on the pen.
- Animals suspected or confirmed of having a notifiable disease in Portugal will be isolated in an isolation pen if the disease has direct transmission, that is, if it is neither Bovine Spongiform Encephalopathy (BSE) nor Enzootic Bovine Leukosis (EBL). According to WOA, Portugal is currently officially free of EBL and has negligible BSE risk.
- Any suspicion of a notifiable disease must be reported by the responsible PBR veterinarian to DGAV (form 1728, sent to: seedspa@dgav.pt and secretariado.lvt@dgav.pt).
- Additional information for each disease can be obtained at the following links:
<https://www.dgav.pt/animais/conteudo/animais-de-producao/bovinos/saude-animal-em-bovinos/doencas-dos-bovinos/>
<https://www.woah.org/en/what-we-do/animal-health-and-welfare/animal-diseases/>
<https://www.cfsph.iastate.edu/diseaseinfo/factsheets/>
- After medical discharge of the cattle, the isolation pen will be carefully cleaned and disinfected with Virkon S (30-minute contact time).

3.2.11.4. Classification of Cattle Suspected / Confirmed with Infectious Disease

3.2.11.4.1. General Rules

CLASS 1: NORMAL HOUSING – GREEN

- Non-infectious diseases or infectious diseases caused by pathogens that are not transmissible to other animal species and with no human infection potential:
 - No fever and no respiratory problems
 - No trauma or infected wounds
 - Pre- and post-operative cattle, without known infectious complications
 - Non-contagious newborn calves

CLASS 2: NORMAL HOUSING – GREEN

- Infectious diseases with low transmission level or caused by non-MRSA bacteria:
 - Wounds infected with non-MRSA bacteria
 - Bacterial pneumonia

CLASS 3: ISOLATION PEN HOUSING – ORANGE

- Infectious diseases caused by multidrug-resistant bacteria or moderately transmissible infectious diseases and/or potentially zoonotic:
 - Fever and/or leukopenia of unknown origin
 - MRSA or multidrug-resistant bacteria
 - Viral respiratory diseases
 - Diarrhoea without fever or leukopenia
 - Diarrhoea in calves
 - Infectious dermatological infections



CLASS 4: ISOLATION PEN HOUSING – **RED**

- Infectious diseases with high transmission level and/or pathogenic to humans. Most notifiable diseases fall into this category:
 - Diarrhoea with fever and/or leukopenia
 - Respiratory diseases with ulcers on oral/nasal mucosa, fever and/or leukopenia, cough, nasal discharge
 - Abortion or perinatal death, or of unknown origin, with fever and/or leukopenia
 - Zoonotic diseases (e.g., *Mycobacterium tuberculosis* complex — *M. bovis*, *M. caprae*, *M. tuberculosis*, brucellosis, anthrax)

3.2.11.5. Special Precautions (Class 3 and 4)

3.2.11.5.1. Movement of High-Risk Patients

- Movement of high-risk patients must be restricted as much as possible.
- Whenever possible, a bovine patient (Class 3 or 4) should be taken directly to the isolation pen, avoiding contact with other areas, animals and people.
- If the patient has diarrhoea, one person should lead the animal, and another should follow with a waste bag to collect faeces. Contaminated areas must be immediately cleaned and disinfected.
- Entry of people into the isolation pen for Class 3 and 4 patients must be minimised and restricted to authorised staff and students.
- All faeces must be removed immediately after medical discharge.

3.2.11.5.2. Diagnostic Testing in Patients Suspected of Class 3 and 4 Infections

- Appropriate samples must be collected and sent as soon as possible to the National Institute for Agrarian and Veterinary Research (INIAV) (<https://www.inia.v.pt/>).
- Biocontainment measures must always be applied by staff and students during diagnostic procedures.
- If the bovine patient requires diagnostic or other procedures (e.g., surgery), these should be performed at the end of the day whenever possible.
- The responsible veterinarian must be consulted before transferring any high-risk patient for diagnostic or surgical procedures, except when clinicians consider the transfer urgent (e.g., critical patient).
- The responsible veterinarian must notify the CHB team of the suspected infectious disease and the biocontainment procedures in place, including cleaning and disinfection (email sent to: biosseguranca@fmv.ulisboa.pt).
- In general, all biocontainment precautions apply whenever the patient is handled.
- Instruments and equipment must be cleaned and disinfected after procedures.
- The responsible veterinarian must ensure that all services involved in procedures are informed about the suspected/confirmed infectious disease and the required PPE.
- The responsible veterinarian is also responsible for ensuring that spaces are properly cleaned and disinfected after procedures, including anaesthetic induction and surgical areas, the recovery room and any other area.
- Whenever possible, surgery in these patients will be performed at the end of the day, after all other patients, except in emergencies.



3.2.11.5.3. Collection of Biological Samples from Cattle Suspected / Confirmed with Infectious Disease

- Biological samples collected from these animals must be properly identified and then placed in a ziplock or whirl-pack bag.
- Biological samples from cattle suspected of zoonosis must be double packaged.
- Care must be taken to avoid contaminating the outside of the bag when placing samples inside.
- The suspected disease or pathogen must be clearly specified on submission forms.

3.2.11.5.4. Special Guidelines for the Management and Care of Cattle with Suspected or Confirmed Infectious Diseases

General

- Strict hygiene and biosecurity are essential for the proper containment of infectious diseases.
- If a bovine presents diarrhoea, one person will lead the animal, and another will follow with a waste bag to collect faeces and then immediately clean and disinfect contaminated areas with Virocid.
- Calves with diarrhoea that require complementary examinations at the Diagnostic Imaging Service are transported in a trailer that will be thoroughly cleaned and disinfected after use.
- Entry into the on-site isolation pen must occur only when necessary.
- Teaching staff and veterinarians may, at their discretion, allow students to access Class 3 patients for didactic purposes (e.g., in cases of salmonellosis or leptospirosis), always under their supervision and with appropriate PPE (disposable white coverall, masks and protective goggles).
- Only teaching staff and veterinarians may attend Class 4 patients.
- Whenever possible, under these circumstances only a limited number of handlers will be assigned to the hygiene of these spaces, and they will not care for healthy cattle in the PBR.
- Biosecurity measures must be complied with by everyone and are displayed on a board located outside the PBR.
- Before and after examining each patient, hands must be washed with soap and water and then disinfected with alcohol gel.
- Surfaces or equipment contaminated with faeces, other secretions or blood must be cleaned and disinfected immediately.
- Special precautions must be taken to prevent environmental contamination by dirty hands, gloves or boots.
- Use the footbaths (Class 3 – Virocid, Class 4 – Virkon S).
- Handlers will clean and change the bedding of the isolation pen once per day, but environmental hygiene is everyone's responsibility; therefore, avoid contaminating the anterooms with straw or manure and assist with general cleaning and maintenance whenever possible.
- Footbaths are refilled daily in the morning after cleaning by handlers and then as needed throughout the day.
- Handlers are responsible for feeding isolated cattle.
- If the patient requires complementary examinations or other procedures (e.g., surgery) that can only be carried out at the Teaching Hospital, these procedures must be performed at the end of the day. Ultrasound examinations, radiographs and endoscopies for Class 3 and 4 patients will be performed in the isolation pen using mobile equipment, which will be cleaned and disinfected after use.
- When Class 3 and 4 patients are isolated, the technical team must clean the “clean” and “dirty” zones of the sanitary barrier area once per day. They must restock any missing item (written



on a board in the clean zone) and ensure that cabinets contain the necessary stock of medications during the animal's isolation.

- A bovine is only allowed to leave the isolation pen, alive or dead, when any suspicion of a notifiable disease has been ruled out. If suspicion of a notifiable disease is confirmed, the animal may only leave the isolation pen after euthanasia and will then be collected by the contracted carcass collection company (ITS|etsa).

Equipment and materials

- Materials must be used exclusively on infected cattle or be discarded.
- If equipment or materials that cannot be discarded were used (e.g., infusion bags, halters/harnesses), these must be cleaned and disinfected before being returned to the Teaching Hospital's Farm Animal Section.
- No equipment or material (dressings, syringes, disinfectant) may be taken into the sanitary barrier area without prior consultation with the responsible veterinarian.
- Partially used medicines must be discarded. They may not be returned (including intravenous fluids) to the Pharmacy.
- Individual material dedicated to the Class 3 or 4 patient is available in a box containing a thermometer, a stethoscope and other materials. The box is kept in front of the isolation pen and must be cleaned and disinfected after medical discharge.

Procedures for entering and leaving the Ruminant Isolation Pen (Classes 3 and 4)

- The following recommendations also apply to all auxiliary services.
- Handlers and the waste collection team must wear specific PPE.
- When entering an isolation pen (Classes 3 or 4) you must:
 - Use the footbath.
 - Apply alcohol gel hand hygiene before entering the isolation pen.
 - Bring all necessary equipment and materials when entering the isolation pen, to minimise traffic in and out.
 - People who handle or examine different patients must discard gloves and other PPE and wash and disinfect hands between patients.
 - Procedures involving highly contaminated sites must be performed last, e.g., rectal temperature measurement, rectal palpation, contact with mucous membranes and abscesses, MRSA-infected wounds.
 - After use, clean and disinfect the material/equipment (e.g., stethoscope, thermometer) with alcohol and place it back in the box (Classes 3 and 4).
- When leaving an isolation pen (Classes 3 or 4):
 - Remove the disposable white coverall and place it in the black biobox.
 - Place disposable gloves and mask (if applicable), sharps and waste in the yellow biobox.
 - If applicable, remove protective goggles, disinfect them with alcohol and hang them again next to the isolation pen.
 - Avoid dragging bedding remnants or faeces into the corridor.
 - Wash boots at the boot-wash station.
 - Wash hands and then use alcohol gel.
 - Use the footbath when exiting the isolation pen.



Diagnostic tests and surgical procedures required in patients with suspected infectious disease

- Diagnostic tests to detect pathogens and zoonotic agents provide essential information for appropriate clinical management of these animals and for the protection of other animals, students and workers. Therefore, it is highly recommended to test cattle whenever there is suspicion of a contagious and/or zoonotic pathogen.
- The responsible veterinarian must ensure that appropriate biological samples are collected and sent to the laboratory under strict biosecurity conditions.
- The CHB must be notified as soon as possible regarding the suspicion of a PBR bovine with a Class 3 or 4 pathogen (email sent to: biosseguranca@fmv.ulisboa.pt).

Dismantling of the Isolation Pen (Classes 3 and 4) prior to disinfection

- Contact the CHB focal point (Eng. Petra Morgado, pmorgado@fmv.ulisboa.pt) immediately after the bovine's medical discharge so that the CHB can supervise, together with the responsible veterinarian, the cleaning and disinfection of the isolation pen.
 - Place all disposable materials in the bioboxes.
 - Seal all bioboxes and leave them in the isolation pen to be collected by the Waste Team.
 - All medical equipment must be immersed in a disinfectant solution for 24 hours before being removed from the isolation pen. It must then be returned to the Farm Animal Section and washed with detergent in the washing machine for 3 hours. The technical team will then carry out additional disinfection and proceed to final storage.
- If another bovine needs to be isolated before handlers are able to disinfect the isolation pen, it must be disinfected by the responsible veterinarian, intern student or technical team.
- After disinfection of the contaminated pen (Class 3 or 4), it must be inspected by the responsible veterinarian, who must approve the procedure before another bovine occupies it.
- For pens that housed adults infected with *Salmonella* spp., the efficiency of the cleaning and disinfection process must be assessed through bacteriological cultures of surfaces. The pen will not be released for a new patient until three consecutive bacteriological surface cultures are negative for *Salmonella* spp. If cultures are positive after the first cleaning/disinfection process, it must be repeated, and cultures must be performed again until three consecutive negative results are obtained.

3.3. Biosecurity Practices in the Farm Animal Ambulatory Service of the Teaching Hospital

These rules aim to prevent the introduction and dissemination of pathogens between farms, between animals, and potential chains of transmission to people, safeguarding public health, animal health and welfare, the institution's prestige and that of its workers and collaborators.

3.3.1. General Clothing and Footwear

- Overalls are the mandatory personal protective garment for off-site practical classes in livestock farms within the curricular units of Epidemiology, Infectiology and Preventive Medicine, Reproduction and Obstetrics, and Farm Animal Clinical Practice and Population Medicine (Farm Animal Ambulatory Service of the Teaching Hospital).
- Overalls must be worn by everyone to mitigate the risk of transmission of pathogens to people or animals, within and between livestock farms.
- Overalls must have the following characteristics: washable overalls, waterproof under moderate rain for 2 hours.



3.3.2. Sequence of Procedures at FMV

- Students must store their personal belongings in the lockers in the General Changing Room.
- Students, teaching staff, clinicians and interns must go to the Garage of the Teaching Hospital Farm Animal Ambulatory Service (HE-EP):
- They must step on a disinfectant-impregnated mat located next to the Garage entrance door in front of the General Changing Room before entering the Garage.
- They must collect overalls provided by FMV, clean and disinfected, exclusive for these off-site practical classes. Personal overalls are not allowed in this area.
- They must collect a pair of washed and disinfected green rubber boots from the boot dryer. The boots are provided by FMV and must be heavy and resistant to protect feet from crushing injuries. Personal rubber boots are not allowed in this area.
- They must disinfect their hands with alcohol gel and proceed to the vehicles.
- When indicated, additional PPE (e.g., masks, face shields, boot covers) will also be provided by FMV.
- Students must bring a thermometer, stethoscope, flashlight, haemostatic forceps and bandage scissors.
- Wearing overalls during travel in the vans is prohibited.
- Eating or drinking is only permitted inside the vehicles or in areas designated by the farms.

3.3.3. Good Biosecurity Practices Upon Arrival at Livestock Farms

- Upon arrival at the livestock farm, students, teaching staff, clinicians and interns must put on overalls, wear rubber boots and disinfect their hands with alcohol gel. This procedure may take place in the farm parking area or in a changing facility, if available.
- Afterwards, boots must be fully immersed in the available footbaths before proceeding to the animal facilities.

3.3.4. Rules of Conduct on Livestock Farms

- Eating or drinking is only allowed inside FMV vehicles or in farm-designated areas (e.g., kitchen, dining room).
- Overalls must be removed during meals.
- Smoking or wearing personal objects (e.g., jewellery, watches, bracelets) during farm visits is strictly prohibited.
- Mobile phone use is limited to clinical records and must be previously authorised by the supervising lecturer. Students must use clean gloves when handling the phone, and its use should be alternated among colleagues.
- Taking photographs or videos requires prior authorisation from the farm manager and/or the supervising lecturer.
- Students must maintain a professional, cooperative attitude and follow the supervising lecturer's instructions.

3.3.5. Good Biosecurity Practices on Livestock Farms

- The use of disposable latex gloves is recommended. Gloves are mandatory when working with calves and adult cows with infectious diseases such as mastitis, pneumonia or enteritis.
- Gloves must be changed between patients and whenever they become dirty or torn.
- Hands must be washed and disinfected with alcohol gel before and after examining each animal (refer to the handwashing protocol).
- Students and teaching staff must wash and brush boots between contact with different groups of animals (e.g., after contact with young animals and before adults) and after high-risk procedures.



- All instruments, including stomach tubes, mouth speculums, thermometers and California Mastitis Test paddles, must be cleaned and disinfected with alcohol after each use.
- Biological samples must be stored in the van in separate compartments, inside insulated containers under refrigeration and properly sealed, until delivery to laboratories.

3.3.6. Good Biosecurity Practices When Leaving Livestock Farms

- Equipment used on the farm must be cleaned, dried and disinfected before being stored again in the van.
- At the end of the visit, students, teaching staff, clinicians and interns must fully immerse boots in the available footbaths.
- Boots must be washed (scrubbed and rinsed) and disinfected with Virocid (Cid Lines) near the vehicles, using an FMV device transported in the vans.
- Overalls must be removed, boots taken off and personal shoes put on. Overalls and boots must be stored in a clean personal bag.
- Hands must be washed and disinfected with soap and water before entering the vehicles.

3.3.7. Good Biosecurity Practices Upon Return to FMV

- Vehicle wheels and sides must be washed and disinfected in the Garage of the HE-EP Ambulatory Service using a high-pressure washer. Students must perform this task under the supervision of the responsible lecturer.

A card must then be placed on the windscreen of each vehicle specifying which disinfectant was used, to ensure the correct contact time (e.g., Aldekol DES FF has bactericidal and yeastcidal activity after 5 minutes of contact time and virucidal activity after 60 minutes).

- Students and teaching staff must remove boots from their bags, brush them to remove organic debris, wash them in the boot-wash station, disinfect them with Virocid (Cid Lines) and place them in the boot dryer.

Students must place the overalls in a black biobox.

Students must wash and disinfect their hands with alcohol gel, leave the Garage, step on the disinfectant-impregnated mat, go to the General Changing Room, collect their personal belongings from the lockers, disinfect their hands with alcohol gel, and leave the General Changing Room.

- Teaching staff must place overalls in a black biobox, wash and disinfect their hands with alcohol gel and leave the Garage.
- Afterwards, student and staff overalls are delivered by a Department of Clinical staff member to the FMV Laundry, where they are washed with appropriate disinfectant in an industrial washing machine and then dried in an industrial dryer.
- Poster 4 summarises the instructions students must follow whenever they have off-site practical classes on farms. This poster is displayed at the entrance of the PBR.

Poster 4

Instructions for students on the Biosecurity Standard Operating Procedures in force during off-site practical classes on livestock farms**OFF-SITE PRACTICAL CLASSES ON LIVESTOCK FARMS****INSTRUCTIONS FOR STUDENTS**

- 1 - Store your belongings in a locker in the GENERAL CHANGING ROOM and lock it with your padlock.
- 2 - Tie back long hair.
- 3 - Bring only a small backpack with 1 notebook and 1 pen or a tablet, a water bottle and an insulated lunch bag with a light meal.
- 4 - Go to the GARAGE located on the ground floor of Building D.
- 5 - Collect overalls from the shelves and a pair of green boots from the boot dryer.
- 6 - Enter the vehicle.
- 7 - Upon arrival at the farm, exit the vehicle with the lecturers, put on the overalls, wear the boots and comply with all biosecurity measures before entering the farm, e.g., disinfect hands and immerse boots in footbaths.
- 8 - Go to the animal facilities, following the lecturers and complying with the farm's biosecurity measures, e.g., passing through sanitary barriers.
- 9 - At the end of the practical class, when returning by the same route, again comply with the farm's biosecurity measures.
- 10 - Leave the farm, wash and disinfect the overalls and boots, remove the boots, take off the overalls, put on your shoes, disinfect hands and enter the vehicle.
- 11 - Procedures 7 to 10 must be repeated whenever practical classes take place on multiple farms.
- 12 - Upon arrival at the FMV Garage, assist the lecturers in washing the vehicle wheels and chassis.
- 13 - Then wash and disinfect the boots and place them in the boot dryer. Disinfect the overalls and hang them on the hangers.
- 14 - Wash and disinfect hands and leave.
- 15 - For more information, scan the QR Code on the poster displayed in the Garage.

NOTE: Ask the lecturers whether eating and drinking are permitted on the farm.

PERSONAL PROTECTIVE EQUIPMENT (PPEs):

- A - Waterproof overalls, green rubber boots and PPE (e.g., gloves, caps and boot covers) provided by FMV.
B - Personal steel-toe boots for Equine Clinical Practice classes.

3.3.8. Special Situations: Pig Farms and Poultry Farms**Pig Farms:**

- Students and teaching staff must shower upon entering the pig farm, and sometimes upon leaving.
- Clothing and boots to be used will be provided by the pig farm.
- Bringing mobile phones or personal items is prohibited.
- Data collection is carried out using clipboards provided by the pig farm.



- Photographic records may only be taken using the lecturer's mobile phone.
- Clinical equipment to be used (e.g., ultrasound scanner, stethoscope) is exclusive to the pig farm.
- Vehicle wheels will be disinfected upon entry to and exit from the pig farm.

Poultry Farms:

- Rules similar to those for pig farms apply, with special attention to the risk of respiratory infections.
- The poultry farm may require the use of FFP2 masks and other PPE during visits taking place in epidemiological outbreak scenarios of Newcastle Disease or Avian Influenza in the region.
- Only poultry farms located in Avian Influenza-free zones should be visited