HANDLING OF LARGE EXPERIMENTAL ANIMALS INFECTED WITH A RISK GROUP 4 VIRUS

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ABSTRACT

The safe experimental handling of animals infected with a new Risk Category 4 virus posed significant biosafety challenges. Novel approaches for restraining, handling and inoculating horses, cats and large fruit-eating bats with equine morbillivirus were developed. Equipment was designed to allow staff working in encapsulating suits access to all parts of a large animal room capable of accommodating seven horses individually. Procedures were developed for the safe handling, anesthesia and euthanasia of experimental animals and for the disposal of infected horse carcasses. Comprehensive staff training for this demanding work included safety consciousness, teamwork and training in first aid and emergency procedures.

INTRODUCTION

Equine morbillivirus (EMV) was first described in 1995 following the deaths of fourteen race horses in training as well as their trainer (Murray et al., 1995; Selvey et al., 1995). The virus has a very wide host range in vitro, replicating well in almost all types of mammalian cells tested. Naturally occurring infections have been described in horses and large fruit-eating bats (Young et al., 1996). As well as these two species, experimental infections have been induced following subcutaneous inoculation of cats and guinea pigs (Westbury et al., 1995) and intracerebral inoculation of suckling mice (B. Eaton, pers. comm.).

EMV infections are lethal; ten of eleven horses and all twelve cats experimentally infected so far by the subcutaneous route have died. Approximately three-quarters of subcutaneously inoculated guinea pigs die. Two of three known human infections resulted in death (Murray et al., 1997). Based on criteria used by the World Health Organization, EMV has been classified a Risk Group 4 pathogen (Australian/New Zealand Standard 2243.3, 1995).

Four species of large fruit-eating bats (genus Pteropus, known locally as flying foxes) are suspected of being the natural hosts for EMV (Young et al., 1996). Because virtually nothing is known of the transmission mechanisms between animal species, experiments were designed to establish basic information. In preliminary experiments, aerosol and close contact transmission mechanisms between fruit bats and horses, horses and horses, and horses and cats were tested.

The handling of a Risk Group 4 agent in all three of these species posed significant hazards for staff. This paper describes the approaches used to maximize staff safety while achieving experimental objectives.

EXPERIMENTAL DESCRIPTION

Room Design

The facility selected for holding large animals infected with a Risk Group 4 pathogen was an air tight room 400 Pa below atmospheric pressure. Adjoining areas varied in pressure from 200 to 300 Pa below atmospheric (Figure 1). The staff and animals entered the room through doors fitted with pneumatic seals. When the room was in the “infectious” mode, staff wearing positive pressure, fully encapsulated, protective suits (Figures 3 and 4), entered the animal area through a chemical shower airlock (Figure 1). Infectious aerosols were removed from the room exhaust air by a tandem filter configuration having an efficiency in excess of 99.999999% (Jamriska et al., 1997).

Breathing quality compressed air for the encapsulating suits was reticulated by two separate systems at 450 kPa. The main system supplied air to the suits via four self-storing, coiled air hoses suspended overhead by polymer-coated stainless steel cables. The suited worker could extend the coiled hose along the cable to its desired position and park
Figure 1: Plan of the animal room and associated areas, showing air pressures relative to atmospheric pressure. Essential aspects of the experimental facility including the geography of the seven horse pens are shown. Entry to the facility was through the north personnel shower air lock (1), the suit change room (2), and the chemical shower air lock (3). The south shower (6) was available only as an emergency exit.

it by applying a small friction brake at the selected position. With 5 m of straight air hose connecting the suit to the coiled hose (Figure 3), the suited worker could access the opposite side of the room, if required. Eighty per cent of the room could be reached in this way. The remainder of the room could be accessed (Figure 1) by the second air system at the north end of the room, which also provided an alternative reserve supply should the main system fail. This reserve allowed four suited workers to exit the room safely without compromising the chemical shower cycle.

Up to seven horses were individually stabled in pens measuring 2.6 m x 2.8 m (Figure 2). Pens were constructed of 40 mm diameter metal rails supported by square 100 mm posts of the same material. To limit the injuries horses might incur by putting their legs through the rails, rubber padding 6 mm thick was secured to the rails up to a height of 1.5 m. This padding also provided protection for workers when standing beside horses.

Contaminated waste was removed during and at the completion of experimentation by sterilization using a double-sided steam autoclave. Large bins containing parts of horse carcasses were processed at 126°C for 7 hours as previous experiments with thermocouples had indicated these conditions were necessary for large loads. After sterilization, all solid waste was incinerated at 800°C. All liquid effluent from the room was collected and batch treated in a pressurized vessel at 125°C for 30 min. prior to being cooled and released into the city sewage system.

Mechanical brushing to remove faecal and other deposits from surfaces was not feasible while wearing protective suits. To facilitate such room cleaning, an industrial high pressure cleaner was used initially to spray detergent over surfaces at 700 kPa, followed by a high pressure rinse at 17 MPa. Testing with a similar detergent, sodium dodecyl sulfate, showed a 2% solution was capable of producing >10⁶ reduction in titre of equine morbillivirus in 3 min. at room temperature. Formaldehyde gas was used for the formal decontamination of the room (Abraham et al., 1996).

The room was constantly monitored by two closed circuit television cameras that relayed images to the facility's remotely-located central monitoring station. This equipment allowed the safety of the workers in the room to be checked and provided a limited capacity to monitor the clinical status of infected animals.
Figure 2: Animal accommodation. Rubber matting was attached to the light-colored rails with plastic ties to provide safety for both horses and workers in suits. Cables suspending the self-storing breathing air lines are visible at the top of the photograph.

Figure 3: Air supply for suits. Four coiled air lines capable of stretching up to 13 m were suspended on four stainless steel wire cables running 80% of the room length. Friction brakes at the ends of each coil allowed the air hoses to be parked at the discretion of the worker without recoil occurring. Five m of soft rubber hosing connected each air coil to a worker’s suit.
Encapsulating Suits

Fully encapsulated, positive pressure suits (model 3506) supplied by ILC Dover (Delaware) were used by all staff entering the room (Figures 3 and 4). Experience from use of this type of suit resulted in the development of numerous "in house" modifications. All suits were fitted with support vests enabling the waist region of the suit to be positioned at a comfortable height for each worker. Areas of the suit fabric or seams showing accelerated wear were identified during routine staff inspections of each suit during showering and after leaving the animal room and reinforced. A portable test apparatus was developed enabling staff to perform weekly pressure decay tests on suits confirming their integrity and adding to overall safety.

Each suit was fitted with its own vortex air cooler unit (3M Co., Missouri) for worker comfort and high efficiency depth type filter module (Matheson model 6134) to ensure protection from infectious aerosols when air supply lines were uncoupled. The pressure drop through the cooler and filter unit resulted in a final suit pressure of 60 Pa relative to room air pressure. The most effective method of mounting the module was direct coupling to the suit (Figure 4). The use of bandolier- or belt-supported modules (Figure 3) proved undesirable for large animal work as constant adjustments compromised the task being done. Each suit was equipped with a radio headset to allow dialogue amongst workers in the room. A "hands free" telephone in the animal room automatically linked a caller with the radio headsets in the suits.

Figure 4: Air filter and air cooling assembly. Compressed air passed firstly through a vortex air cooler assembly (part W-2862, 3M Co., Missouri) and then through a high purity 0.3μm depth filter housed in a stainless steel canister (Matheson model 6134, ) before entering the back of the worker's suit. The weight of the air filtration/cooling assembly is borne by a reinforced pad glued to the suit.
Decontamination of the suits on leaving the room was achieved by a chemical shower consisting of a 3 min. water spray for the cleaning of suits followed by a 4 min. spray with 3% Lysol (Reckitt and Coleman, New Jersey). This decontaminating agent had been shown previously to produce >10^6 reduction in EMV titres when analyzed in an in vitro cell culture system. A final 1 min. water spray removed the decontaminant chemical from the suits. Should the chemical shower procedure fail, an emergency manually-operated spray containing 1% glutaraldehyde was available.

Suits used in large scale animal work show more wear and tear than their laboratory counterparts, having an average life span of 12-16 weeks when used daily. The necropsy of horses on the floor was a particularly stressful task for suits, probably because of the frequent bending of the body.

**General Aspects of Animal Work**

To maximize safety, animals were selected for ease of handling and having quiet dispositions. Suppliers, with established reputations, provided bats from a captive colony and cats and horses that were known to be easy to handle. Placing cats and horses in close proximity for disease transmission studies meant that the placid temperaments of all animals were important to minimize stress. All procedural aspects of animal experimentation were approved by the institute animal ethics committee.

The encapsulating suits made clinical monitoring of animals difficult and apart from heart rate and rectal temperature measurements on horses, clinical assessments were restricted to observations of water and food consumption, respiratory rates, general appearance, behavior, and faeces consistency.

**Horse Accommodation and Handling**

Large animals such as horses have the capacity to injure workers by violent movements, and damage suit integrity exposing workers to the biohazard. Thus, each horse pen was fitted with a ratcheted crushing device for horse restraint (Figure 5). These crushes were used for daily animal handling including assessments of heart rate, rectal temperature measurement, nasal or subcutaneous inoculations, bleeding from the jugular vein and the injection of sedatives and anesthetics. Prior to such handling, horses were sedated by xylazine (1 mg/kg) administered by intramuscular injection.

![Figure 5: Crush mechanism for horse restraint. A crush device fitted with ratchets at both ends restricted horse movements. This device provided safety both for the suited worker and the horse during handling.](image-url)
Use of suits and two pairs of gloves meant that heart rate monitoring by auscultation or palpation was not possible. A heart rate monitor (Dynafit Pulsetronic, HRM10) similar to those used by athletes was modified for use with horses. Hair was closely clipped from either side of the horse and ultrasound gel applied to improve monitor sensitivity. The first component of the monitor was a transducer transmitter mounted on a curved stainless steel plate designed to conform to the shape of the horse’s thorax and placed over the heart. A handle was attached to the plate to allow easy positioning. The second component was a handheld digital receiver having a communication range with the transmitter up to 1 m.

The clinical conditions of horses were followed carefully by daily examination and by monitoring with closed circuit cameras. Horses in the terminal stage of disease were euthanased by injecting xylazine (1 mg/kg) intravenously to allow the safe use of a captive bolt device while the horse was still standing. If a horse was already on the floor, the captive bolt device could be used only if the horse was motionless because of the risk to personnel. To ensure worker safety in this potentially hazardous environment, stunning with the captive bolt was followed immediately by exsanguination by severing the vessels of the neck to ensure certain death. Exsanguination had added safety aspects: (1) the blood could be washed away before necropsy thus reducing the risk of slipping; and (2) critical organs were not covered in blood making observations clearer and dissections safer.

Laboratory rules required three experienced staff in suits to be present for horse necropsy examinations. Only one at a time was permitted to handle sharp cutting instruments, and the cutting motion was to be away from the body whenever possible. The primary requirement for three workers allowed rest periods during specimen collection and cutting the carcass, and the sharing the physical workload.

Animal body parts were placed in 100 L bins with lids for steam sterilization at 126°C for 7 hours in the animal room autoclave. Tissue specimens for laboratory analysis were placed in primary containers which were sealed, decontaminated with 1% glutaraldehyde, and placed inside secondary containers that were sealed and decontaminated with the same chemical disinfectant prior to removal from the room.

**Handling Procedures for Fruit Bats**

Large fruit-eating bats of the genus *Pteropus* (commonly called “flying foxes” because their heads resemble those of foxes) occur naturally in northern Australia, along the eastern seaboard, and in southeast Asia and southern Africa. Mature animals weigh up to 1 kg and have a wingspan up to 0.8 m. (Figure 6). In addition to very sharp teeth and claws, they have sharp spurs on the joints of their wings giving them alternative options for defense or suspending themselves upside down when cornered. The experimental handling of these aggressive animals posed significant hazards for workers in suits and so routine handling methods had to be replaced by novel approaches.

Figure 6: Large fruit bat. The natural pose for these animals is to hang upside down from the top of the cage. Although passive in this photograph, animals become aggressive when approached, spreading their wings and displaying sharp teeth and claws.
Animal ethics advice was that two or more bats must be caged together. Because the bats’ natural posture was to hang upside down from the roof of the cage, a simple crush mechanism, as used for restraining cats (Figure 8), was not effective. To minimize the possibility of damage to suits by animal attack, a strict policy of anesthesia before experimental handling was adopted. As the caging facilities did not allow injectable drugs to be used safely, general anesthesia was administered by inhalation of gaseous Halothane M&B (Rhone Merieux, Australia). This anaesthetic was found to be superior to an alcohol, ether and chloroform mixture (ratio 1:2:3), ether alone or chloroform alone. Enclosure of the bat cage was achieved with a perspex board underneath the animal and a clear plastic cover over the top and sides of the cage. Gaps between the perspex and the cover were sealed with tape. Halothane was administered by a compressed air-driven nebuliser placed inside the enclosure. After a sufficient depth of anesthesia had been achieved (about 5-6 min.), the cover was removed and the bats removed using thick Kevlar gloves to protect the rubber gloves of the worker. The bat was then wrapped in a towel and placed on an examination board and restrained by a taut rubber shroud while inoculation, bleeding or other experimental procedures were completed (Figure 7).

Figure 7: Anaesthetized bat. General anesthesia was induced by Halothane gas before animal handling. Protective gloves were worn over the normal rubber gloves and animals were wrapped in towels and then restrained under a rubber flap attached to the examination board.
Bats were euthanased by an overdose of carbon dioxide administered after the cage was sealed as described above. Carbon dioxide was found to be a quick, safe agent for this purpose and was acceptable to the Animal Ethics Committee.

Handling Procedures for Cats

Domestic cats posed hazards for suited workers because of their sharp claws and teeth. Prior to experimental handling, they were restrained by a simple crush mechanism consisting of a false back to the cage which was drawn forward thus immobilizing the cat against the front wall of the cage (Figure 8). In this position, the cat could be anaesthetized and inoculated safely. For safety reasons, cats were not handled after inoculation and temperature monitoring and other clinical examinations were not attempted. Cats were anaesthetized by an intramuscular injection of xylazine (2 mg/kg) and ketamine (15 mg/kg) and euthanased by carbon dioxide, a method resulting in fewer artifacts for subsequent pathological examination of tissues than from barbiturates.

![Figure 8: Cage crush mechanism for cat restraint. A false back to the cage attached to a frame allowed the cat to be moved to the front of the cage and restrained while being inoculated or sedated.](image)

Staff Biosafety Training and Procedures

Only staff with significant experience in handling infected experimental animals were considered for training for work in suits with a Risk Group 4 agent. All members of this team were required to pass a medical examination showing the absence of conditions such as diabetes, uncontrolled epilepsy, angina and respiratory insufficiency which would have precluded suit work. All were required to complete a First Aid course including cardiopulmonary resuscitation with supplied oxygen so that emergency aid could be provided for any staff member collapsing while inside the animal room. First aid equipment included a finger pulse oximeter (Nonin Medical, Minnesota) that could provide a pulse rate when applied to the finger of a gloved hand, and an oxygen resuscitation unit (Laerdal, Norway).

Emergency procedures for handling a collapsed staff member in a suit were difficult to devise because of the inevitable compromise between immediate aid for a distressed staff member and the likely
exposure to a potentially life-threatening virus. In brief, if a worker collapsed or fainted, external help would have been sought immediately. All scenarios considered cannot be described here, but opening the suit in the animal room was a last resort option for life-threatening situations. If an emergency occurred, the laboratory had an emergency rescue team specially trained in the hazards associated with this work.

Training in suit/air line awareness and familiarization with all other equipment and installations were critical issues for staff safety. Having two and sometimes three people working in a large room with many animal cages and enclosures required each to be aware of the position of other workers and their trailing air lines. Removal of unused cages, equipment and waste was an essential part of creating space for safe movements within the room.

Access to the animal experimental facility was restricted to fully trained staff by use of numerically pass-coded electric latches at the entry door. Work was always done in pairs, and when particularly hazardous work was necessary, such as inoculations and necropsies, three staff worked together. Radio communication was particularly effective, especially for necropsy procedures with large animals. Direct connection of the telephone line to the radios of all staff in the room also contributed to safety by allowing discussions with colleagues outside the animal room.

A contingency plan was developed in case of accidental exposure of staff to the biohazard. This consisted of a two stage plan where staff experiencing a relatively low level of exposure were isolated on site and monitored daily for the development of any clinical signs of illness. If a major exposure occurred, the staff member would have been transferred immediately to the infectious diseases unit of a large metropolitan hospital and chemotherapeutic treatment with the drug, ribavirin, started promptly. This drug had been shown to have antiviral activity against EMV in cell cultures (P. Selleck, pers. comm.). However, the demonstration of comparable effects in vivo have not been completed.

Weekly meetings of all experimental staff and senior management were held to discuss experimental design changes, procedural modifications, equipment assessment and staff safety issues. These meetings were a valuable forum for the many staff involved to discuss and coordinate issues evolving during experimentation and so achieve a greater overall safety.

DISCUSSION

As procedures for the safe handling of horses, cats and fruit bats in biosafety level 4 facilities have not been described, novel solutions to biocontainment problems had to be developed. Appropriate room design was essential to allow freedom of movement for all suited workers to all parts of a large room. Staff safety was significantly enhanced if breathing air lines were controlled appropriately. Preliminary experience showed suspension of breathing air supply hoses on overhead steel cables provided this flexibility in large animal rooms.

Cats were accommodated singly in cages for these experiments and this arrangement worked satisfactorily. However, for social reasons, a policy of two cats per cage was adopted and this compromised safety aspects of handling if both animals did not respond identically to the anaesthetic. Subsequent experience showed that cats were content to be in a cage by themselves as long as they could see another cat close by.

The types of animal handling and examination permitted were substantially restricted to reduce the risks to staff safety. Devices that could transmit body temperature readings by telemetry were tried in bats and guinea pigs but were not reliable to use. Rectal temperature measurement of horses was the only temperature monitoring method considered safe. Bleeding of animals was considered a hazardous procedure and was not attempted for infected or potentially infected cats.

The safety limitations of working in suits meant that clinical information about the health status of infected animals was somewhat limited. Such compromises needed to be negotiated carefully to establish an appropriate balance between the scientific objectives of the experimentalists, animal ethics requirements, and the biosafety concerns of laboratory managerial staff. In general, it was agreed that observations of water and feed consumption, respiratory rates, general appearance, behavior and faeces consistency were sufficient for clinical assessments where temperature measurements were not possible.
Damage to suits was considered to be the major biosafety concern for workers. Procedures were designed to minimize the use of sharp objects in the room, but because of the need to bleed animals and to do necropsies, sharps instruments could not be avoided entirely. Before each experiment, a sharps audit was done routinely to identify any edges or other protrusions on cages or other installations likely to be hazardous to suits.

Emergency procedures for staff working in suits in large animal rooms were particularly difficult to develop, especially should a worker collapse. Oximeter devices were available to allow heart pulse rates to be measured through rubber gloves. External assistance was available from a trained emergency rescue team aware of the particular hazards associated with a Risk Group 4 agent. The consensus agreement was that a worker’s suit could be opened in the animal room only in life-threatening situations where further determinations were necessary concerning breathing or the need for resuscitation. If there was concern about the immediate welfare of a collapsed worker, then staff were advised to consider the risk from the infectious agent to be the lower concern.

REFERENCES


