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Production and management of specific pathogen free and gnobiotic animals

Kuladip Prakash Shinde and Shailesh Kumar Gupta

Division of Livestock Production and Management, ICAR-National Dairy Research Institute, KARNAL (HARYANA) INDIA (Email: kuls164@gmail.com; sgshailesh786@gmail.com)

Specific pathogen free animals are those animals which are free from a group of particular pathogen but these animals are not necessarily free from other organism, which are not specified, in the conducted experiment. Gnotobiotic: A word derived from the Greek "gnotos" and "biota" meaning known flora and fauna. An animal stock or strain derived by aseptic cesarean section (or sterile hatching of eggs) which are reared and continuously maintained with germfree techniques. According to International Committee on Laboratory Animals (ICLA) "Specific Pathogen Free (SPF) animals, which are free of specified micro-organism and parasites but not necessarily free of on the ones which are not specified." Synonyms of SPF animals are –Disease free animals, healthy animals, pathogen free animals, clean animals, caesarian derived animals. Historically, the concept of gnotobiotic experimentation is credited to Pasteur's efforts in 1885 Nonhuman primates are important for disease investigation, therapy and vaccination (Schmidt, 1972 and Dormant *et al.*, 1990). Within the past 20 years there has been a widespread interest in the artificial rearing of animal removed from the dam at or near the end of gestation and keep isolated from conventionally reared animals. There are two primary reasons for producing animals under such conditions. The 1st of these is to break the cycle of the some of the infectious disease organism present commonly transmitted from animal to animal. The 2nd is to provide more uniform experimental animal for many phase of basic and applied research by reducing one of the variables diseases. It has been estimated that approximately 20 million animals are being used for testing and are killed annually; about 15 million of them are used to test for medication and five million for products. Different factors may may affects experimental animals which results in change in experiments (Melby, 1983; Small, 1983). China has become one of the biggest country using lab animals and highest number of lab animals are found in China.(e.g., specific pathogen free; genetically modified) increasingly used in scientific research-16 million a year, compared to 12 million in the 25 European Union countries in 2005. People for the ethical treatment of animals (PETA) reported that the National Centre for Laboratory Animal Sciences (NCLAS) in Hyderabad, supplies approximately 50,000 animals to laboratories every year.

Use of specific pathogen free and gnotobiotic animals: These animals are used in certain conditions of body which become severe by secondary complication. Ex. Wound. In India, among rodent group of animals e.g. mice, rat, G. pig, rabbit, mice are predominantly used in most of cases followed by others. SPF animals are very much useful in experiments which are carried out for longer period.

Mouse: Most frequently used. Pharmacology, genetics of mammals, virology, models of human diseases.

Rat : Physiology of cognitive processes, behaviour, models of diabetes.

Rabbit : Serology, insulin quantification, pyrogens quantification,.

Guinea-pig : In microbiology and serology, physiology of the auditory system.

Hamster : Genetics.

Frog : Physiology of blood circulation, electrophysiology.

Fish : Molluscs, insects.

These animals are used in studying of defense mechanism of the body. These animals are used in studying the ageing process of individual in animals and human being. In body relationship between different microflora can be study by using the specific pathogen free and gnotobiotic animals. These animals may be used as the steril organs and tissue for different investigations and research. Different diet related researches and its reaction can be estimated by using these animals.

Purchase and techniques for the SPF and gnotobiotic animals :

Before the purchasing these animals we should get the important information about these animals. We should select a defined specific pathogen list for the stock. Different diagnostic and detection methods should apply during the purchasing. Previous screening and screening test organization should be cleared. By the use of history

the Surveillance programme and disease history should be carefully monitored.

The principle is that obtain animals from a stage in their life cycle when they are either a minimum number of contamination or not at all.

Caesarian technique : The placenta acts as a very efficient filter and prevents the fetus from becoming infected with most bacteria, virus and parasites. The object is to remove and free from the conventional pathogens.

– Normal parturition is delayed by giving daily injection of progesterone to the mother during the last three days of pregnancy.

– In case of bird fertilized eggs can be passed directly from the outside via the germicidal trap, to the interior where hatch normally.

– The pregnant dam is prepared for surgery by removing the hair from abdomen by shaving.

– In case of small animals like rat and mice cervical dislocation is followed which is quick and humane. For large spp. Halothane/oxygen mixture can be used for anaesthesia. Hysterectomy used for obtaining young from the dam.

– Gravid uterus is put in a sterile plastic box which containing some sterilizing will kill microorganism. The uterus is opened dries the fetuses. The fetal membranes removed, leave the placenta in contact at the umbilicus for a short time while respiration has been initiated.

– Young one usually requires stimulation by gentle with sterile gauze and drying of the nostril.

– Food is given by hand day and night until they are already inside the barrier.

Hand rearing : The animals maintained at a temperature 33-35^{0}C and the humidity kept at 50 per cent or higher The hand rearing of new young like mice is difficult. So we can fed the new born mice with the help of rubber nipple or stomach tube which stimulated to pass urine and faeces.

Foster nursing : The foster nursing of surgically derived pups is possible in a barrier room. We should introduce a number of good foster mother, who should be mated so as to deliver 1-3 day prior to the date of surgery. The new born young should put into warmed receptacle containing fostering mother. After half an hour foster mother is removed from the litter and put with the new young ones.

Care and management

– Discipline of the whole operation of SPF unit is that of preventing reinfection and invasion of the clean area by pathogen. A persons working inside should be clean because they act as carrier for many diseases.

Staff : A person should be in good health, active, intelligent and well trained. Smoking eating drinking and eating should be prohibited in all working area. Person working with this should be well aware and well understood of the operations aspects of the unit. Personnel are required to take a shower while entering and then don sterilized garments including hair nets, face masks, and jump suits. Personnel facilities include staff and record room, sufficient changing room, decontamination area and first aid.

Boot cleaning and disinfection : Visible organic material may be removed from boots. Boots may be disinfected by soaking in a clean bath of an appropriate disinfectant . It is important to frequently empty, clean, and refill the boot bath to prevent it from being contaminated with organic matter. Disposable boots may be used.

Training for SPF animal care and use : All SPF animal care and use personnel must be trained on SPF protocols. All SPF trained personnel must follow the SPF care and use guidelines at all times.

Veterinary rounds : The veterinary staff makes regular rounds through the facility to observe the animals, their housing conditions and husbandry procedures. All animals in SPF care are observed daily by an animal care staff. Each area of the facility is also assigned a veterinary technician and an area veterinarian.

Decontamination of the room : The room should be properly sealed and left over night after fumigation and thereafter should be properly ventilated The different methods adopted by animal houses depend upon the facilities available, the cost, the simplicity and efficiency of the procedure. When liquid formalin is used 1 ml of 10 per cent solution for every cubic foot of the room space is required. The room temperature should be at least 18^{0}C with relative humidity about 80 per cent. The sterilization of all consumable is very essential Formaldehyde gas may be produced by exothermic reaction. Usually two parts of formalin are added to one part of crystals of potassium permanganate. When liquid formalin are added to one part of crystals of crystals of potassium permanganate.

Animal room : Animal room should be protected against ingress by pets such as wild rodents and insects. Holes created should be sealed. Adequate arrangement should be provided to the receipt or incoming animals. Baker (1979) recommended a noise intensity of 85 db.Animal brought into an animal house should not put at risk animals which are already there. In cage rearing system housing temperature may affect ed by the nesting materials and type of choosen animals (Woods, 1980 and Corning, 1992). Where surgery is to be performed suitable operation

facilities should be provided including separate preparation area for the animals, equipments and staff. There should be post operative recovery area. The relative humidity of the laboratory animals should around 50 per cent and ranges between of 40-70 per cent (Clough, 1987).

Food and bedding stores should be clean dry vermins and insect free. In addition food stores should be cooled and sunless provided with ventilation. Perishable food should be stored in cool room refrigerators and freezers.

Floors should be moisture resistant, non-absorbent, impact resistant, and relatively smooth, although textured surfaces may be required in some high-moisture areas and for some species.

A vermin free collection area should be provided for waste prior to its disposal. Special arrangement should be made for handling carcasses and radioactive or other hazardous materials.

Each animal room is emptied, cleaned and fumigated with formaldehyde and water vapors at least once per year so as to prevent the buildup of bacterial contamination. The fumigation is carried out by evaporating a mixture of formaldehyde and water to near dryness by boiling. In rat and mouse house half a liter of formalin (40% formaldehyde) and one liter of water is allowed for each 1000 ft.

Inside the SPF unit :

Laminar flow hood (*LFH*) : A unit which provides a sterile work environment by very high efficiency filtration of the air that circulates across the work surface. Room air is taken in through the back of the hood and passed through the HEPA filter. Sterile air moves across the work surface from back to front and is expelled through the sash opening.

MI cage/unit : A housing unit consisting of a polycarbonate shoebox-style bottom, stainless steel wire-bar lid and a polycarbonate top that holds a permeable filter. Additional items also include bedding, water bottle, food, and cage card holder.

Mobile shelf-unit (*MSU*) : Mobile shelving unit and cover used to temporarily store and transport clean micro isolator cage units.

Bath : The polycarbonate cage bottom located in the laminar flow hood which contains the disinfectant solution. If the bath becomes cloudy or excessively soiled with feces or bedding, the solution should be emptied in the sink and box rinsed with tap water.

Introduction of animals : Specific-pathogen-free (SPF) rats and germ-free mice were purchased; the animals inside their delivery box passed into the breeding unit through the 'dunk' tank and there allowed to breed.

Dispatch of animals : Weanling animals, other than breeding replacements, are not kept in the rodent breeding unit, but are transferred to a stock room in an experimental unit. A litter is transferred by placing them in a sterile cardboard box which is then sealed into a polythene bag and passed out through the 'dunk' tank.

The litter then enters the experimental unit through a further 'dunk' tank, where the polythene bag is removed and the litter caged. The transport boxes are stored flat and made up as required.

Biosecurity : Good biosecurity begins with personal cleanliness. Showering or washing facilities and supplies should be provided, and personnel should change their clothing as often as necessary to maintain personal hygiene. Personnel should not be permitted to eat, drink, apply cosmetics, or use tobacco in animal facilities. Visitors should be limited as appropriate, and institutions should implement appropriate precautions to protect the safety and well-being of the visitors and the animals. It is essential that the agricultural animal care staff maintain a high standard of biosecurity to protect the animals from pathogenic organisms that can be transferred by humans. Disposable gear such as gloves, masks, coats, coveralls, and shoe covers may be required under some circumstances. Personnel should not leave the work place in protective clothing that has been worn while working with animals.

Ventilation : Ventilation, humidity, temperature, lightening and noise contribute for good science. High level of Ammonia causes the respiratory problems in the rodents (Lindsey *et al.,* 1978). In coming air should be filtered from dust particles, when most sources are also removed. Ultraviolet pathway within the dunk tank can be used. Temperature should be maintained between 10 to 21^{0}C. Number of air changes 5 to 15/ hr. provided adequate ventilation. Heat is removed from the exhaust air by means of a heat pump unit incorporated in the extract system and used to heat the water in the breeding unit. Light intensity is very important for the laboratory animals because it may influence the aggression and cannibalism in the animals (Fall, 1974; Weihe, 1976). Belhorn (1980) suggested a light intensity level of 323 lux (30 fc) for animals care and management practices.

Materials of biological origin : Materials of biological origin such as cells, tissues, serum and cultures will be damaged or destroyed by autoclaving or gas sterilization techniques. Therefore, they must be tested before they are introduced into animals. Please contact Rodent Health Monitoring.

Stem : Most commonly used method of sterilization carried by means of double autoclave situated in the barrier with one door.

Sterile diets : Diets are autoclaved and ethylene oxide fumigation. Water is decontaminated by acidification, hyper chlorination and/or filter sterilization,. Sometimes nutritive value destroyed but sterilization with ionizing radiation and microwave infrared is healthy.

Screening and control of pathogen : Regular routine sampling of stock for bacteria and parasites is done.

Serological examination : Each SPF room will have serology conducted every 3 months. The serum test will be done annually by a basic panel. The infected animals will be immediately treated or discarded.

Routine screening procedures : Swabs from the surface of the walls and floors of the breeding unit and samples of the filtered water supply and of the 'dunk' tank fluid are taken four times at weekly intervals. These are examined for the number and type of organisms present. *Salmonella, Mycobacterium, Bordetella, Pasteurella, Mycoplasma and Corynebacterium.*

Hazardous wastes : That are toxic, carcinogenic, flammable, corrosive, reactive, or otherwise unstable should be placed in properly labeled containers and disposed of as recommended by occupational health and safety specialists. In some circumstances, these wastes can be consolidated or blended.

Record keeping : Record keeping is important for-

– Animal house plans which includes typical floor plans, all fixtures etc.

– Breeding stock, purchases and sales records.

– Minutes of institute animals ethics committee meeting.

– Records of sick animals.

– Death records.

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