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**OVERVIEW**

**Compton. PCR and RT-PCR in the Diagnosis of Laboratory Animal Infections and in Health Monitoring, pp. 458-468**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

**SUMMARY:**Polymerase Chain Reaction (PCR) has become a mainstay in the diagnosis of, and surveillance for infectious diseases in laboratory animals due to its speed, sensitivity, and specificity. Depending on the agent and context for testing, it has either replaced or complemented serological/microbiological methods. PCR from animal samples usually detects active infections, whereas environmental samples detect both active and past infections. It is essential to know the site of infection and route/ease of transmission for the agent of interest, so that the correct samples are collected.  PCR is helpful in cases where the agent cannot be cultured in vitro, or if serology is not possible due to a lack of species-specific secondary antibodies. Some zoonotic agents (e.g., Streptobacillus moniliformis, Mycobacterium bovis) that can pose a risk to staff when cultured are safer diagnosed with PCR. PCR sampling is often consistent with the 3R’s, as it came be minimally or non-invasive to animals (e.g., fur or oral swabs, feces, plenum, sump, or other environmental samples).

Some pitfalls of PCR include its high sensitivity, which can lead to false-positives due to minute nucleic acid contamination. For example, germ-free mice fed autoclaved diets may still test falsely positive for bacterial rRNA, thus microbiological techniques for surveillance in the germ-free context is still essential. Its high specificity can be a disadvantage for agents (e.g., RNA viruses) that mutate quickly, leading to false-negatives. Nucleic acids, particularly RNA, must be handled carefully and processed quickly to avoid degradation prior to testing.

**QUESTIONS**

1.  Which of the following agents are not readily, or unreliably detected, in soiled-bedding sentinels? (Choose all that apply)

a.   MNV

b.   Helicobacter hepaticus

c.  Filobacterium rodentium

d.  Pneumocystis carinii

e.  Murine astrovirus-2

f.  Radforia affinis

2.  LDEV can be readily detected as a contaminant in biologics using all of the following but:

a.  MAP

b.  Viral culture

c.  PCR of cells from contaminated cell line

3.  Detection of pathogens in exhaust air dust exposure is dependent on \_\_\_\_\_\_\_\_\_\_\_.

**ANSWERS**

1.  c, d, f

2.  b

3.   The amount of infectious agent released into the airflow, IVC rack type/location of air filtration and sampling, duration of exposure/when the rack was last thoroughly decontaminated.

**Bahadoran et al. Importance of Systematic Reviews and Meta-analyses of Animal Studies: Challenges for Animal-to-Human Translation, pp. 469-477**

Domain 3 - Research

SUMMARY: This article discusses the importance of systematic reviews and meta-analyses of animal experiments due to the contention that research using animals provides a low level of evidence for human prognostic and therapeutic studies, further begging the question – are animal experiments necessary for research associated with human health?

The following is a summary of each of the sections this article discusses:

Pros and Cons of Animal Experiments: This section discusses the benefits of using animal models in their areas of use, as well as the pros and cons of their use. It is summarized in the following bullets and tables:

* Areas of Animal Experiments

o    Basic and Applied Research

o    Testing of drugs and other products

o    Educational purposes

* Benefits

o    Making possible genetic manipulations

o    Studying effects of newly developed drugs

o    Understanding disease pathophysiology

|  |  |
| --- | --- |
| **PROS** | **CONS** |
| Have led to several medical advancements for humans | Therapeutic efficacy in animals does not often translate into clinical practice |
| Have led to new hypotheses | Expensive |
| Provide an opportunity to investigate:   * The toxicity and safety of new compounds and treatments * The mechanistic actions of drugs, discovering new drug targets and biomarkers * A complete life cycle * Environmental and genetic manipulations, which are not feasible in humans * Human biology, pathophysiology, and the underlying mechanisms of all diseases * Novel targets for clinical treatments | Differences in response to newly developed drugs leaning into adverse human effects undetected in animal studies |

Systematic Review and Meta-analysis: Clinical Versus Preclinical Point of View

Definitions:

* Systematic Review: A study that provides a transparent mean for gathering, synthesizing, and appraising the findings of studies on a particular topic or question by using scientific methodology. They summarize a large body of evidence and explain differences among studies with the same question.
* Meta-analysis:The statistical method used to combine results from relevant studies, and he resultant larger sample size provides greater reliability of the estimates of a treatment effect. Considered the top of the hierarchy of evidence.

Systematic reviews of animal studies have not been commonly done compared to clinical research and there is an emphasis that systematic reviews and meta-analyses of experimental investigations can clarify whether and how translation from animal to clinical research could progress and allow for evaluation of the appropriateness of the animal models.

Applications and Usefulness

* Clinical Trials

o    Meta-analyses conducted to estimate overall effect sizes of specific interventions

o    Critical value for decision making in clinical practice

* Preclinical Studies

o    More exploratory

o    Used to address gap in knowledge and generate hypotheses

o    Can be used to help design clinical trials

o    Provide insights for clinical studies that help the translational process

o    Addressing weaknesses and improving the quality of future animal studies (study design, reporting, ethical issues)

o   Determine the current extent of knowledge in the field

o    Provide new insights for unanswered questions

o    Minimize unnecessary duplication of animal studies

o    Minimizes biases from individual studies when combining with other studies

o    Provides good estimation of the power of the reported effect size and reproducibility of results

o    Combines findings from previous experiments

o    Improve the methodologic quality of animal experiments, evidence-based selection of animal models, evidence-based translation of animal date to the clinic, and implementation of 3Rs to conduct more ethical animal experiments

o    For Meta-analyses: Increases power of analysis

Key Steps to Conducting A Systematic Review of Meta-Analysis:

|  |  |
| --- | --- |
| **Step** | **Explanation** |
| Framing the question | * Forming a research question consisting of participants, interventions, comparisons, outcomes, and study design |
| Defining eligibility criteria | * Specifying study characteristics, such as length of follow up * Specifying report characteristics, such as years of publication |
| Transforming the research question into a search strategy | * Using key words or terms and Boolean operators including ‘or”, ‘and’, or ‘not’ |
| Searching databases and collecting references | * Identifying appropriate databases and sources of studies and checking other sources, including reference lists * Saving references in a reference manager software |
| Screening and selecting studies | * Unifying citations into one file in the references software and remove duplicates * Initial screening of titles and abstracts of the references and identifying papers based on potential relevance * Retrieving full texts in potentially relevant papers * Assessing full-text reports for compliance of study with eligibility criteria * Making final decisions on study inclusion and proceed to data collection |
| Extracting Data | * Extracting information including details of methods, participants, setting, context, interventions, outcomes, results, publications, and investigations |
| Assessing the quality of the included studies | * Assessing quality of study report using published scales (e.g. Jadad scale, Newcastle-Otawa scale) |
| Conducting meta-analysis on extracted data | * Determining the likely nature of outcome data (dichotomous, continuous, ordinal, counts, and rates, and time-to-event data) * Calculating effect size for each comparison * Weight the effect sizes * Calculating a summary of effect size * Calculating heterogeneity * Deciding whether random-effect meta—analyses, fixed-effect meta-analyses or both methods will be used |

Common Limitation and Pitfalls of Systematic Reviews and Meta-analyses of Animal Studies for Translation Purposes

* Intrinsic limitations of animal models (e.g. the diversity of animal species studied, differences of experimental designs, and variations in animal characteristics)
* Poor design and reporting of animal studies

o    Lack of randomization, allocation concealment, blinding of researchers to treatment, and blind assessment assessments of outcomes  may lead to overestimation of treatment effects

o    Low sample sizes

* Factors limiting the applicability of systematic reviews and meta-analyses of animal studies

o    Heterogeneity of data (due to variations in animals (including age, sex, and species) and study design and methods)

o    Publication bias: The tendency on the parts of investigators, reviewers, and editors to submit or accept manuscripts for publication based on the direction or strength of the study findings

o    Methodologic weaknesses in systematic reviews and meta-analyses (i.e. poor methodologic features – non-explicit testable hypothesis, lack of assessment off publication bias and methodologic quality, study quality, and heterogeneity

o    Potential misunderstanding in meta-analyses

Potential Steps Toward Solving These Problems

* Better reporting of animal experiments
* Providing more transparency regarding animal studies
* Conducting high-quality systematic reviews and meta-analyses of animal data

QUESTIONS

1. Which of the following describes a systematic review?

a. Compares patients who have a disease or outcome of interest with patients who do not have the disease or outcome and looks back retrospectively to compare how frequently the exposure to a risk factor is present in each group to determine the relationship between the risk factor and the disease.

b. Analyzes data of variables collected at one given point in time across a sample population or a pre-defined subset.

c.   Answers a defined research question by collecting and summarizing all empirical evidence that fits pre-specified eligibility criteria.

d.   Samples a cohort (a group of people who share a defining characteristic, typically those who experienced a common event in a selected period, such as birth or graduation), performing a cross-section at intervals through time.

e.  The use of statistical methods to summarize the results of a collection of studies that answer a specific research question, in order to determine overall trends

2.  Which of the following describes a meta-analysis?

a.  Compares patients who have a disease or outcome of interest with patients who do not have the disease or outcome and looks back retrospectively to compare how frequently the exposure to a risk factor is present in each group to determine the relationship between the risk factor and the disease.

b.  Analyzes data of variables collected at one given point in time across a sample population or a pre-defined subset.

c.    Answers a defined research question by collecting and summarizing all empirical evidence that fits pre-specified eligibility criteria.

d.  Samples a cohort (a group of people who share a defining characteristic, typically those who experienced a common event in a selected period, such as birth or graduation), performing a cross-section at intervals through time.

e.   The use of statistical methods to summarize the results of a collection of studies that answer a specific research question, in order to determine overall trends

3. Put the following in order to highest to lowest in the evidence hierarchy:

1. Case-control studies
2. Cross sectional studies
3. Animal trials and *in vitro* studies
4. Meta-analyses
5. Case reports
6. Systematic Reviews
7. Cohort studies
8. Randomized clinical trials

ANSWERS

1. c

2.  e

3.  d, f, h, g, a, b, c, e

**Abad Cobo et al. Anesthesia Protocols used to Create Ischemia Reperfusion Myocardial Infarcts in Swine, pp. 478-487**

Domain 3: Research K3. animal models (spontaneous and induced) K8. experimental surgical techniques and instrumentation

Primary Species: Pig (*Sus scrofa*)

SUMMARY: NOTE: Ischemia Reperfusion Myocardial Infarct = IRMI

The porcine model of ischemia-reperfusion is one of the most commonly used models. Selecting an anesthesia protocol that has minimal influence on infarct size is also crucial. Previous studies postulated that using volatile anesthetic agents such as halogenated gases such as sevoflurane, or opioids such as remifentanil may protect against ischemia-reperfusion injury. Therefore, the use of those agents in this model of cardiac injury remains controversial.

# Premedication, Sedation, And Analgesia

*Ketamine*: Ketamine was used as a sedative for anesthetic premedication in most anesthetic protocols reviewed. Doses used in the publications reviewed ranged from 5 to 30 mg/kg intramuscularly, alone or combined with other drugs, and is commonly combined with benzodiazepines such as diazepam or midazolam. . Ketamine can also be combined with other sedatives such as **α**2 agonists (for example xylazine, medetomidine or dexmedetomidine) to extend the duration of its sedative effect to approximately one hour and improve its sedative and analgesic effects in animals that are difficult to manage.

*Tiletamine-Zolazepam*: Tiletamine-zolazepam is chemically similar to a combination of ketamine and a benzodiazepine. The initial sedative effect of tiletamine is faster than ketamine; however, it seems to produce greater respiratory depression and leads to longer anesthetic recovery times. In pigs, it can produce an initial state of excitement. Effects at the cardiovascular level are more pronounced than those of ketamine, especially with regard to increasing the heart rate and therefore myocardial oxygen consumption. It has been used in several studies of the porcine model of acute ischemia-reperfusion myocardial infarct but seems to be associated with higher mortality rates.

*Midazolam*: Midazolam is a short-acting benzodiazepine that exerts its action by stimulating the GABA receptor GABA. Midazolam was found to be used in several studies assessed in this review, either as a sedative or as a continuous infusion. However, it can prolong recovery times. In some animals, midazolam may lead to a period of excitement if used alone. Therefore, it is administered to previously sedated animals. Midazolam is mainly used as an adjunct due to its limited effects on the cardiovascular and respiratory systems. It provides a good degree of hypnosis and muscle relaxation, allowing a reduction in the doses of other drugs used at induction and also reducing the necessary minimal alveolar concentration (MAC) of inhalational anesthetics such as sevoflurane or isoflurane by up to 50%.

*Fentanyl and Remifentanil*:They provide an excellent analgesic effect, and due to their rapid onset of action, they can be used as “rescue” analgesics. They cause dose dependent respiratory depression. Therefore, we recommend that they are used with mechanical ventilation or ventilatory support. Fentanyl and remifentanil potentiate the effects of other drugs such as alfa2 agonists and benzodiazepines as they can reduce their doses by 40 to 70 %. Its prolonged use at high doses can increase peripheral vascular resistance due to the release of vasopressin. At high doses, it can increase mean arterial pressure and reduce cardiac output.

*Alpha 2 Agonists*: At low doses, these drugs can be useful as sedatives and analgesics when administered just before the infarct modelling process. Because of their hemodynamic characteristics, these drugs might be dangerous after infarct creation. However, with adequate monitoring and the use of selective drugs such as dexmedetomidine at low doses of 1 to 2 µg/kg, a good analgesic level can be achieved. Xylazine is less selective than other drugs of the same family, such as medetomidine and dexmedetomidine.

*Acepromazine*: Acepromazine is a phenothiazine that has neuroleptic action. It exerts its activity by blocking the dopaminergic receptors as well as the peripheral α-adrenergic receptor. Pigs are commonly treated intramuscularly. Maximal sedation appears at 30 to 40 min after administration, is dose-dependent, and may last 3 to 4 h. Acepromazine has a poor analgesic effect that can be compensated by jointly administering opioids.

# Induction

*Propofol*: Propofol is a hypnotic agent that exerts its action on GABA receptors. Due to its high lipid solubility, it has an ultra-fast effect with a short duration of 3 to 5 min. Administration of propofol with a benzodiazepine and an opioid is a common combination. As has been described in other species, propofol produces a severe depression in breathing, causing periods of apnea mainly after rapid administration as a bolus or at high doses. Thus, the use of mechanical ventilation is recommended.

*Thiopental*:It is primarily used for anesthetic induction prior to endotracheal intubation due to its rapid action at doses of 15 to 20 mg/kg in animals without premedication and 7 to 12 mg/kg in previously sedated animals. It is stored in the fatty tissue and has a cumulative dose-dependent effect. At the cardiovascular level, thiopental, and drugs from the same group (e.g., pentobarbital) decrease arterial pressure,98 producing compensatory tachycardia, which increases myocardial oxygen consumption. As a compensatory effect, it also decreases coronary vascular resistance and increases myocardial blood flow, potentially leading to ventricular arrhythmias.

# Maintenance

*Isoflurane and Sevoflurane*:The blood concentration of these drugs depends on blood/ gas solubility, cardiac output, and the difference in the drug’s partial pressure between the alveoli and blood. Administering these drugs to animals that have not been previously sedated can cause agitation, nervousness, apnea, and hypoxemia. High concentrations of isoflurane irritate the airways and produce cough. Although induction with sevoflurane is smoother and less irritating, it is still only recommended for use in depressed animals or when vascular access is not available. In the porcine model of acute myocardial infarction of the ischemia reperfusion type, sevoflurane maintenance produced better hemodynamic stability than did isoflurane, based on mean arterial pressure. Frequency of ventricular fibrillation was 81% in swine anesthetized with isoflurane compared with 52% in pigs anesthetized with sevoflurane.

*Antiarrhythmic Adjuvants*: When creating myocardial infarcts in pigs, antiarrhythmics are usually used to prevent ventricular arrhythmias, with lidocaine and amiodarone the most commonly used drugs. Also, oxygenation of myocytes during diastole is greater and does not decrease cardiac output.

*Lidocaine*:In continuous infusion, lidocaine was found to decrease MAC, thereby decreasing the need for inhalational anesthetics.68 Lidocaine exerts anti-inflammatory and analgesic effects in species such as dogs, cats, and horses. However, despite having an antiarrhythmic effect in pigs, lidocaine does not appear to decrease the MAC of halogenated anesthetics and its analgesic effect is much lower.

*Amiodarone*:A dose of 5 mg/kg administered as a slow intravenous bolus can be administered in continuous infusion at 150 to 300 mg/h. In a swine model of ventricular fibrillation, administration of amiodarone with adrenaline did not improve defibrillation efficacy and caused hypotensive effects. Extravasation of injectable drug formulation produces tissue necrosis while oral administration of high doses before the procedure can lead to greater hypotension and predispose patients to arrhythmias during the induction period.

# Discussion: This review revealed the scarcity of articles that specifically report the anesthesia-related mortality that occurs in association with creating ischemia-reperfusion myocardial infarcts in pigs. The articles that were identified showed high heterogeneity in their anesthesia protocols. In many studies, animals that die during the model development process are replaced and therefore are not counted as experimental deaths. In these studies, the mortality rate is determined only at the end of the study.

In the studies we included (Table 1), the average mortality rates associated with the creation of the model ranged from 10% to 60%. Furthermore, nearly all studies used female swine. Hormonal influences on the evolution of acute myocardial infarction in human clinical cases has been described and may be present in the porcine model.

The mortality rate of the model can be influenced by anesthetic reactions such as malignant hyperthermia, which is more frequent in swine than other species but is nonetheless rare. Another possible cause of mortality in this model that is not directly associated with anesthesia protocol is the individual variability of coronary anatomy, specifically the existence and distribution of collateral branches of the left anterior descending coronary artery.

The main limitation of this study is the difficulty of carrying out an exhaustive review in light of the great variety of studies that use ischemia-reperfusion injury and the difficulty of finding publications that report mortality rates during the creation of the infarct, prior to carrying out experimental procedures or other interventions.

In the literature review, the authors found that tiletamine displays more acute cardiovascular effects than those observed after ketamine administration. A higher mortality rate has been reported when tiletamine is used for preanesthetic sedation. In addition, the mortality rate also varies among studies that use inhaled isoflurane or total intravenous anesthesia for anesthetic maintenance. Among the studies selected in this review, 3 had a slightly higher mortality (33%); these studies used tiletamine-zolazepam, pentobarbital or thiopental.

Pentobarbital depresses myocardial contractility and accentuates hypotensive effects, and the combination tiletamine zolazepam seems to cause more hemodynamic instability than similar combinations such as ketamine midazolam. However, the studies we reviewed did not show an increase in mortality rate due to induction time of myocardial ischemia. A recommended balanced anesthetic protocol could include premedication with 10 to 30 mg/kg ketamine in combination with an opioid and benzodiazepine to obtain an adequate sedation plane.8 Propofol might provide stable cardiovascular induction. After the induction period, a low dose of a selective α2 agonist such as dexmedetomidine (0.5 to 1 µg/kg) may be useful to decrease heart rate and compensate for the relative hypotension induced by propofol. However, the animal should be monitored for bradycardia. Although maintenance with sevoflurane could increase cardiovascular stability, further studies are needed to determine the effect of these drugs on infarct size. In addition, the halogenated dose may be reduced with a continuous infusion of midazolam, causing a more neutral effect at the level of the myocardium and a lower cardioprotective effect. Continuous infusion of antiarrhythmics such as lidocaine or amiodarone can also effectively prevent the occurrence of ventricular arrhythmias.

QUESTIONS

1. T/F: In swine, the lesion is created by introducing an angioplasty balloon through the femoral artery and occluding the first branch of the anterior descending coronary artery.

2. The typical duration of ischemia for IRMI in swine is

1. Between 2 and 5 minutes
2. Between 5 and 20 minutes
3. Between 40 and 150 minutes
4. Longer than 150 minutes

3. T/F: Yucatan pigs require less ketamine and midazolam for sedation.

4. T/F: In swine and other species ketamine has a cumulative effect.

5. T/F: In swine, benzodiazepines have a major role counteracting ketamine muscle rigidity.

6. T/F: In pigs, ketamine at high doses (above 25 to 30 mg/kg) can cause tachycardia and sensitize the myocardium to the action of endogenous catecholamines.

7. Tiletamine-zolazepam is administered at a:

1. 1:1 ratio
2. 2:1 ratio
3. 1:3 ratio
4. 3:1 ratio

8. T/F: Fentanyl and remifentanil have minimal effects at the cardiovascular level.

9. T/F: Fentanyl has an ultrarapid onset of action and a shorter duration than femifentanil

10. With alfa2 sedatives, hypertension normalizes, or hypotension develops after:

1. 10-13 minutes
2. 1-3 minutes
3. 3-5 minutes
4. 15-18 minutes

11. T/F: At high doses, xylazine can sensitize the myocardium to the action of the endogenous catecholamines, resulting in arrhythmias.

12. T/F: the so-called propofol infusion syndrome (Acidosis, refractory bradycardia, myocardial failure, rhabdomyolysis, asystole, and death) has been described in swine after continued administration for periods that exceed 48h, at doses greater than 4 mg/ kg/h. The use of propofol in cardiac surgery has been questioned due to its depressant effect on vascular resistance and myocardial contractility.

13. T/F: Amiodarone has far fewer adverse effects than are reported for other antiarrhythmic agents, so it is used to treat ventricular arrhythmias of unknown origin or patients who are refractory to other treatments.

ANSWERS

1. F (it is the second branch)

2. c

3. F (they require a bit more)

4. T

5. F (The effect is minimal in the porcine species compared with other species)

6. F (Although it has been shown in dogs)

7. a

8. T

9. F (the opposite is true)

10. c

11. T

12. F (it has been described in humans)

13. T

**ORIGINAL RESEARCH**

***Reproduction***

**Stone et al. A Nonsurgical Embryo Transfer Technique for Fresh and Cultured Blastocysts in Rats, pp. 488-495**

Domain 3: Research

Primary Species: Rat (*Rattus Norvegicus*)

SUMMARY: Nonsurgical embryo transfer (NSET) methods have been developed, the first developed in mice (mNSET). The mNSET device transfers unmodified blastocysts, and blastocysts that have been genetically modified by introduction of ES cells, pronuclear injection, or lentiviral gene transfer. It has also been used to test effect of culture conditions on post-implantation development of mouse zygotes cultured to blastocyst stage, test toxic effects of various compounds on post-implantation embryo development, and maternal dietary requirements to study effects of high fructose diet and zinc deficiency. It has also been used for transcervical sperm transfer and in a Chlamydia infection model. The mNSET device is not transferrable to rats, so rNSET devices still require research. In contrast to pseudopregnant mice, the cervix of pseudopregnant recipient rats is NOT dilated at the optimal time of embryo transfer of blastocysts. Administration of oxytocin is needed to dilate cervix. This study used a Teflon catheter (length 37 mm; diameter 0.71 mm)and hub attached to a 2 uL pipette to deliver cultured blastocyst stage embryos and fresh embryos into the uterine horn of pseudopregnant female Sprague Dawley or Fischer 344 rats. The rNSET groups were compared to surgical embryo transfer groups in terms of pup recovery, body weight, and fecal corticosterone responses. The rNSET is an effective alternative to SET in terms of blastocyst stage embryos rather than fresh cultured. No significant difference in fecal corticosterone was noted between the SET and rNSET groups. The animals also did not exhibit weight loss, other than a transient drop in weight in the anesthesia groups.

QUESTIONS

1. What is given to rats to dilate their cervix prior to embryo transfer?
2. What hormone is typically measured as an indicator of stress in rodents?

ANSWERS

1. Oxytocin
2. Fecal corticosterone

***Husbandry***

**Pallas et al. Compressed Paper as an Alternative to Corn Cob Bedding in Mouse (*Mus musculus*) Cages, pp. 496-502**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY:The paper is primarily focused on the comparison of compressed paper (CP) as an alternative to corncob (CC) bedding. CC has been used in laboratory rodent facilities because of its minimal cost, high absorbency, biodegradability, and reduction of intra-cage ammonia levels. Unfortunately, CC is able to be eaten by rodents and can cause rats to spend significantly less time in slow wave sleep pattern. Additionally, CC can act as a fomite for potential pathogens such as parvovirus. The authors hypothesized that CP bedding would have greater absorptive capacity than CC and that housing mice on CP bedding would be a procedural refinement by decreasing early cage changes and by lowering intracage ammonia concentrations. A wide variety of genotypes and strains were used for this study (B6, CD1, BALB/c and 129). Total absorption assay, immediate absorption assay, intra cage ammonia concentration was measured via a handheld gas meter, and colony wide assessment of spot change frequency was performed. The absorbency of CP compared to CC bedding revealed that CP absorbed significantly more water than CC at both 5 and 60 minute contact times. The frequency of early cage changes individually ventilated cage (IVC) containing CP and CC bedding was recorded for 13 rooms and revealed that CP bedding had a significant decrease in cage changes compared to CC bedding cages. The average intra cage ammonia concentrations in IVC were significantly lower in CP than in CC at various time points after the cage change. The number of mice in cage significantly influenced ammonia levels. In static caging systems, the ammonia levels were similar between CP and CC. The investigation identified that CP bedding has higher total and immediate absorbent capacities than CC. Moreover, in this study CP decreased the amount of early changes needed during a cage change cycle and lower ammonia levels in ventilated cages compared to CC. Therefore, CP bedding has potential to improve the welfare of the mice by reducing the frequency of early cage changes and by decreasing inadvertent effects on research data.

QUESTIONS

1. Free response: What is the source of ammonia in caging systems?
2. The Permissible Exposure Limit for ammonia set by OSHA averaged over an 8 hours workday is?
   1. 25 ppm
   2. 50 ppm
   3. 75 ppm
   4. 100 ppm
3. Which of the following are altered by phytoestrogen compounds found in corn cob bedding?
   * 1. Fecal production
     2. Learning and Memory
     3. Splenic size
     4. Uterine function

ANSWERS

1. Ammonia is produced in mouse cages when urease-positive fecal bacteria come into contact with mouse urine.
2. b. 50 ppm
3. d. Uterine function

**Baker. Cage Position and Response to Humans in Singly-housed Rhesus Macaque (*Macaca mulatta*), pp. 503-507**

Domain 4: Animal Care

Primary Species: Macaques (*Macaca spp*)

SUMMARY: The purpose of this study was to determine whether rhesus macaques housed in bottom tier caging showed poorer responses to offered positive human interactions (hand feeding of treats) than those housed in upper caging. Poor response to offered human interaction might suggest that humans are a greater stressor for bottom-tier housed animals than top tier housed animals.

Bottom tier caging is thought to be less desirable for diurnal, arboreal NHP species because it tends to have lower light levels and offers no opportunity for vertical escape. Studies on the welfare impact of bottom tier caging on NHPs have been inconsistent in their results.

Analysis of data from hand-feeding of treats to study animals showed no relationship between cage location and response to feeding attempt. Length of time animal animals had been housed in indoor caging did positively relate to willingness to take treats, which suggests that animals more habituated to indoor caging are more willing to take treats from humans. Age also positively correlated with increased treat acceptance, indicating that animals become more accustomed to positive reinforcement with time.

Female animals responded more poorly to humans in two areas: they took treats less often and they showed more fear behaviors. No relationship was identified between animal behavior and proximity to the room door.

QUESTIONS

1. Which of the following factors made animals less likely to accept a treat from a human on this study?
   1. Increasing age
   2. Increasing amount of time in indoor housing
   3. Male sex
   4. Female sex
2. T/F: Cage position (top tier vs. bottom tier) was significantly associated with treat acceptance in this study.

ANSWERS

1. D. Female sex
2. False

***Management***

**Luchins et al. Cost Comparison of Rodent Soiled Bedding Sentinel and Exhaust Air Dust Health-Monitoring Programs, pp. 508-511**

Domain 4: Animal Care

SUMMARY: Soiled bedding sentinel health monitoring programs have been traditionally used to detect rodent pathogens in rodent facilities.  Due to limitations of soiled bedding sentinel programs and potential benefits (e.g. reduction in animal numbers, increased sensitivity, and decreased animal care staff labor) of exhaust air dust health monitoring, several institutions have considered exhaust air dust health monitoring as a replacement and/or adjunct to traditional soiled bedding monitoring programs.  The authors analyzed/evaluated the annual costs of both health monitoring programs.  Based on their evaluation, the exhaust air dust health monitoring program had the following advantages over the soiled bedding sentinel program: it was 26% less expensive; it decreased staff time spent on health monitoring activities, and it replaced the use of live sentinel animals.

QUESTIONS

1. Exhaust air dust PCR testing has been found to be effective at detecting which of the following agents?
2. Mouse hepatitis virus
3. Mouse norovirus
4. *Helicobacter* spp.
5. Pinworms
6. All of the above
7. True or False: The total annual diagnostic testing cost was significantly lower for the exhaust air dust program.

ANSWERS

1. e. Sendai virus, astrovirus, lactate dehydrogenase virus, *Rodentibacter pneumotropicus* and *R. heylii*, fur mites, and enteric protozoa also mentioned in the article
2. False; the total annual diagnostic costs were similar for both programs

**Lin Teoh et al. Construction of an Affordable Open-Design Recirculating Zebrafish Housing System, pp. 512-518**

Domain 4: Animal Care

Primary Species: Zebrafish (*Danio rerio*)

SUMMARY: The use of Zebrafish as a vertebrate animal model has increased tremendously in various fields of biomedical research in past decade. Although easy to produce and use, maintaining them in large numbers becomes realistically impossible particularly in the small academic institutions with limited resources due to costly commercially available housing setups. Teoh et. al., demonstrates that a functional zebrafish facility can be built in an easy and affordable manner. Authors displayed that such a facility could be built in a short span (3 days) from scratch with readily available resources with minimal tools and expertise. The cost of twelve 6 L fish tank zebrafish facility constructed in this study was approximately $750.00 USD. The constructed system could hold up to 360 fish in 12 tanks with a housing density of 5 zebrafish/L. In summary, authors have constructed a simple and affordable housing system for a small laboratory.

QUESTIONS

#### In the present study, what stocking density (zebrafish/ Liter) of zebrafish authors recommend for their system?

1. 4 to 6
2. 4 to 10
3. 8 to 12
4. 3 to 5
5. In the below diagram, what structures are indicated by the Red arrows and what is their function?

A picture containing text, indoor, kitchen appliance

Description automatically generated

1. Bio balls, for filtration system
2. Bio rings, for filtration system
3. Bio rings, for enrichment
4. Bio balls to indicate Hardness of water

ANSWERS

1. b
2. b

***Anesthesia***

**Gergye et al A Comparison of Ketamine or Etomidate Combined with Xylazine for Intraperitoneal Anesthesia in Four Mouse Strains, pp. 519-530**

Domain 2: Management of Pain and Distress

Tasks: (1) Recognize pain and/or distress, (2) Minimize or eliminate pain and/or distress, (3) Administration of anesthesia

Primary Species: Mouse (*Mus musculus*)

SUMMARY:  KX anesthesia is one of the intraperitoneal (IP) anesthetic protocols most widely used in mice, but the mixture is known to produce results that vary depending on the sex and/or strain of the animals used, and the type of experiment being performed.  Some studies have reported a continued response to noxious stimuli after standard dosing of KX administered IP, and higher doses of KX have been associated with higher mortality rates.  Additionally, KX administered IP may introduce experiment confounding effects due to the anti-inflammatory effects of ketamine on the immune system, which include reduced function of cytokines, lymphocytes, neutrophils, and NK cells.  Ketamine usage has also been associated with increased tumor metastasis in cancer research.  Etomidate has seen some veterinary usage as an alternative to ketamine, but its use in mice has not been widely studied. The purpose of this study was to evaluate etomidate-xylazine (EX) anesthesia as an alternative to ketamine-xylazine (KX) when administered IP.  Male and female mice from four different strains (Crl:CD1(ICR), C57BL/6NCrl, BALB/cJ and NU/J) were given a single IP dose of ketamine 100 mg/kg + xylazine 10 mg/kg or etomidate 20 mg/kg + xylazine 10 mg/kg, then evaluated for induction, sedation depth and duration, whether or not surgical anesthesia was achieved, and possible side effects. Physiologic parameters monitored included respiration rate, systolic blood pressure, rectal temperature, and acidosis levels.

All mice exhibited marked hypothermia under sedation (despite being placed on a heating pad) and hypothermia was especially significant in NU mice regardless of treatment (causation attributed to the NU phenotype).  No overall differences were seen between KX and EX with respect to lowest respiration rate, lowest systolic blood pressure, lowest rectal temperature, or levels of acidosis, but lowest heart rates with EX were significantly higher than with KX- these results suggest that EX and KX have similar safety profiles.

Several significant sex- and strain-dependent physiologic differences were noted when comparing sexes or individual strains:

* + Across all strains, surgical anesthesia was achieved in 44% of EX mice compared with only 4% of KX mice
  + BALB/c (79%) and NU (87%) mice given EX were significantly more likely to experience an adverse hyperexcitement response during induction than other strains
  + C57BL/6 mice were significantly more likely to achieve surgical anesthesia when given EX (94%) or KX (18%) compared to other strains, and only EX in this strain produced reliable surgical anesthesia (overall rate of 93%, 100% in males, 87% in females) among all test groups
  + Except for C57BL/6 strain mice, females in all strains were more likely to reach surgical anesthesia than males
  + Overall, females had slightly lower sedation times for a given anesthetic treatment than did males
  + Mice that received EX had shorter sedation times (43 ± 24 min) than mice given KX (52 ± 15 min), and CD1 mice exhibited much shorter sedation times than other strains (e.g., only 22 min sedation with EX)
  + Mice given EX had longer recovery times (6.5 ± 10.9 min) compared with mice given KX (2.6 ± 4.8 min)

Authors conclude that EX administered IP is an equally effective sedative for mice compared to KX.  At doses tested, EX was found to be a more effective surgical anesthetic than KX, although the durations of surgical anesthesia were short and not consistent for any strain except C57BL/6 mice. Given the high rate of hyperexcitement and inconsistent surgical depth seen test groups, results of this study suggest EX would be best suited for use in C57BL/6 mice to achieve consistent levels of surgical anesthesia for 15 to 20 min with a low risk of hyperexcitement on induction. Further study is needed to optimize EX for use in multiple mouse strains.

QUESTIONS

1. Which strain of mice used in this study was significantly more likely to achieve surgical anesthesia when given EX or KX compared to other strains?

a. CD1

b. C57BL/6

c. BALB/c

d. NU

2. Which 2 strains of mice used in this study were significantly more likely to experience an adverse hyperexcitement response during induction? (choose  2)

a. CD1

b. C57BL/6

c. BALB/c

d. NU

3. T/F: According to this study, EX administered IP is an equally effective sedative for mice compared to KX?

4. T/F: According to this study, EX was found to be a more effective surgical anesthetic than KX?

5. T/F: According to this study, females had slightly lower sedation times for a given anesthetic treatment than did males?

ANSWERS

1. b. C57BL/6

2. c. BALB/c (79%),  d. NU (87%)

3. True

4. True (although the durations of surgical anesthesia were short and not consistent for any strain except C57BL/6 mice)

5. True

**West et al. Intraperitoneal Alfaxalone and Alfaxalone-Dexmedetomidine Anesthesia in Sprague-Dawley Rats (*Rattus norvegicus*), pp. 531-538**

Domain 2: Management of Pain and Distress (administration of anesthesia)

Primary Species: Rats (*Rattus norvegicus*)

SUMMARY

Introduction: Injectable anesthetic methods, such as the most common formulation of ketamine-xylazine, can lead to unpredictable and variable anesthetic states in rodents. The advantages of injectable methods include lower initial cost, no need for anesthetic equipment, batch surgery potential, the ability to provide multimodal anesthesia, and ease of use with certain procedures (e.g., oral surgeries). Alfaxalone is commonly used in veterinary medicine due to its wide margin of safety, smooth induction and recovery, painless injection, and absence of tissue reaction if extravasation occurs. Previous studies have determined that alfaxalone IV can achieve surgical anesthesia in rats; the authors of this study sought to determine the anesthetic effects of IP administration for 1) alfaxalone only and 2) alfaxalone-dexmedetomidine. They secondarily sought to determine ideal doses for both male and female rats.

Materials and Methods: Alfaxalone alone (3 dosages) and in combination with dexmedetomidine (4 dosages) were tested IP in male and female Sprague-Dawley rats (total n = 44). All animals remained on room air. Time to induction (LORR), induction quality (e.g. spontaneous movement), anesthetic duration, HR, RR, temperature, and time to recovery were recorded. Physiologic parameters and anesthetic depth parameters were noted concurrently every 5 minutes; anesthetic level was tested with the pedal withdrawal reflex in combination with an in-house response scoring system. After 60min of anesthesia, saline (alfaxalone only) or atipamezole (alfaxalone-dexmedetomidine) were administered.

Results: All doses of alfaxalone alone achieved a sedative level of anesthesia only. All doses of alfaxalone-dexmedetomidine achieved surgical anesthesia. Induction quality for both groups was smooth and physiologic parameters remained stable. Both treatment groups showed sex-associated differences: females maintained longer respective sedation/anesthesia than males.

Hypoxia (<90% SpO2) was noted in the alfaxalone only group for females.

Discussion: For 60 min sedation of female rats, 20mg/kg alfaxalone is the recommended IP dose. For 60 min surgical anesthesia in female rats, the authors recommend 30mg/kg alfaxalone and 0.05mg/kg dexmedetomidine IP. Dosages for male rats were not determined in this study and need further evaluation, as the dosages tested were not adequate for consistent sedation/anesthesia respectively. Overall, the alfaxalone-dexmedetomidine combination provided more consistent anesthesia with more comparable results in males and females than alfaxalone only.

QUESTIONS

1. Alfaxalone is a…
   1. Neuroactive steroid anesthetic (Schedule III)
   2. Neuroactive steroid/GABA agonist (Schedule IV)
   3. Neuroactive steroid/GABA antagonist (Schedule III)
   4. Neuroactive steroid/GABA antagonist (Schedule IV)
2. T/F: Alfaxalone causes minimal cardiovascular and pulmonary effects, but care must be taken with titration dosing, as metabolites will accumulate with repeated administration.

ANSWERS

1. b
2. F – Alfaxalone has rapid elimination, allowing for repeated dosing without accumulation. It is true that it causes minimal cardiovascular and pulmonary effects.

**Ambar et al. Anesthetic Effects of Intramuscular Alfaxalone-Ketamine in Naked Mole Rats (*Heterocephalus glaber*), pp. 539-545**

Domain 2: Management of pain and distress; Admin of anesthesia

Tertiary Species:Other Rodents

SUMMARY

* NMR (*Heterocephalus glaber*) are members of the family Bathyergidae, are subterranean rodents and native to sub–Saharan Africa. They are Eusocial and can live in colonies of up to 300 animals. Possess unique physiology that make them good models of aging due to longevity, cancer resistance studies, hypoxia resistance studies (due to their low basal metabolic rate, high hematocrit and Hgb with high 02 affinity) and in somatosensory research due to unique characteristic of pain perception. Also used in behavioral studies due to unusual social structures – including endocrine and neuronal regulation of reproduction
* Chemical immobilization necessary for examinations and diagnostics, but inhalational via facemask or induction box unsafe and unreliable for NMRs. Ketamine + xylazine used with little information as to variables it can induce.
* Alfaxalone can be administered IV, IM and SQ and is a neuroactive steroid molecule that acts on GAVA receptors, resulting in centrally mediated muscle relaxation and anesthesia. Has a similar profile to propofol and effects may be potentiated when the drug is combined with other sedatives and analgesics. Addition of ketamine (NMDA antagonist) provides deeper level of anesthesia and some analgesia.
* This study used 5M + 5F and alfaxalone dosed at 4mg/kg IM + Ketamine at 20mg/kg IM into thigh muscle.
* Results: median time to induction = 180s, anesthesia duration = 60min and time to recovery was 18min. Males showed longer induction times, shorter duration, and no difference from females in recovery times. HR and RR significantly decreased, and loss of all withdrawal reflexes.
* Conclusions: AK regimen was safe and effective method for brief immobilization of NMR, with short, light anesthesia in males, and a longer and deeper anesthesia in females.

QUESTIONS

1. NMR in captivity require high or low humidity
2. T/F: NMR obtain all their water requirements from tubers/ diet
3. What is the scientific name of the Damaraland mole rat (hair mole rat) and are they eusocial as well or not?

ANSWERS

1. High humidity (50-60%)
2. True
3. *Fukomys damarensis*, and yes, they are also a eusocial animal

**Andrews et al. A Comparison of Buprenorphine, Sustained-release Buprenorphine, and High-concentration Buprenorphine in Male New Zealand White Rabbits, pp. 546-556**

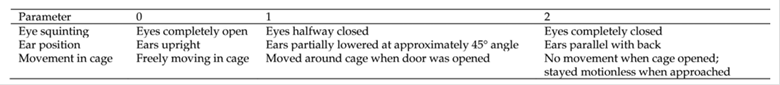
Domain 2: Management of Pain and Distress; T2: Minimize or eliminate pain and/or distress

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY: Pain management in rabbits, as in any prey species, is considered challenging. They hide their symptoms and are often stressed simply by handling for injections or other treatments. While long-acting medications do go a long way towards reducing the number of restraint episodes and the associated stress, adverse effects of opioids in rabbits can be life-threatening (e.g., gastrointestinal ileus and anorexia). In addition, adverse effects of sustained-release or high-concentration buprenorphine in other animals include tissue reactions and sterile abscesses at the site of injection. In the veterinary field, three formulations are available for use: an injectable, FDA-approved formulation; a compounded sustained-release formulation; and a high-concentration formulation FDA-approved for cats. This paper sought to compare the pharmacokinetics and side effects of the three formulations when given subcutaneously in rabbits, specifically their effect on GI motility, behavior, and local tissue reaction at the injection site. Overall, no injection site reactions were observed grossly in any rabbit. Rabbits who received the sustained-release formulation (BupSR) had evidence of partial anorexia during days 1-3 of administration, and those given the high concentration (BupHC) had reduced food consumption on days 2-3. Two rabbits given the BupHC formulation had neurologic signs following drug administration, which in one rabbit were severe enough to warrant euthanasia. Overall, the paper concludes that buprenorphine and BupSR are likely useful in the private practice or research settings, but BupHC should be used with caution due to the potential for neurologic adverse effects.

QUESTIONS

1. What type of an opioid analgesic is buprenorphine?
2. What does this represent?



ANSWERS

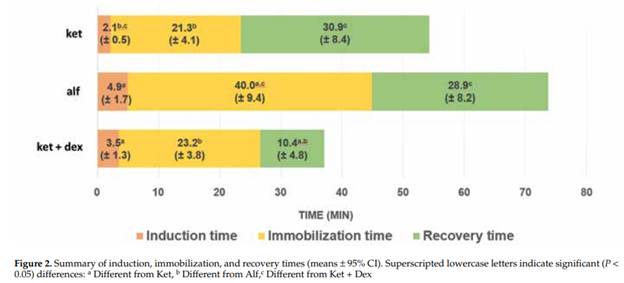
1. Partial µ agonist, δ and κ antagonist
2. Grimace scale (modified)

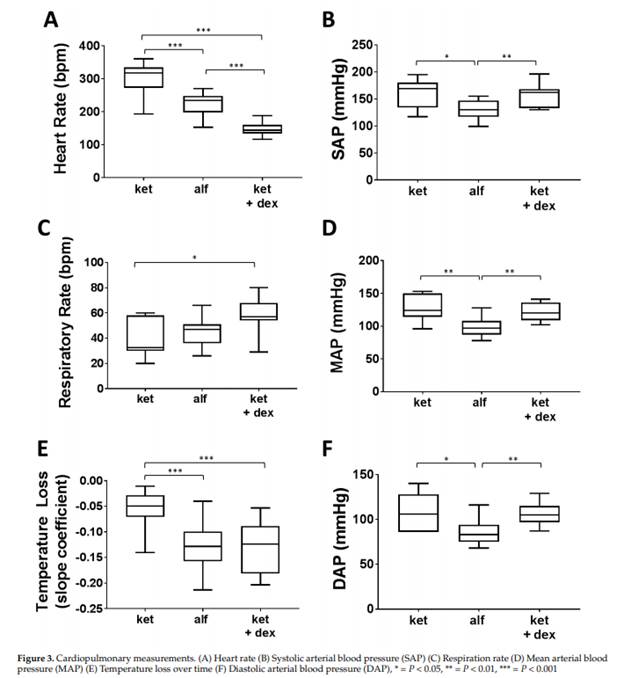
**Finnie et al. A Comparison of the Efficacy and Cardiopulmonary Effects of 3 Different Sedation Protocols in *Otelemur garnetti*, pp. 557-566**

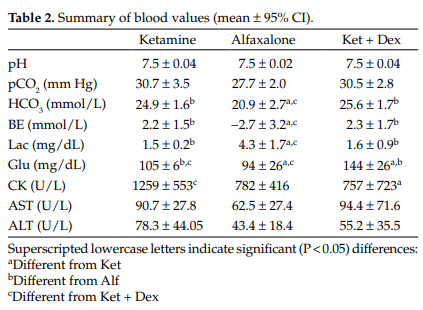
Domain 2: Management of Pain and Distress

Tertiary Species: Other Nonhuman Primates

SUMMARY: This article tested three sedation protocols in 34 Otolemur garnettii: 1) alfaxalone (8 mg/kg IM; 11 animals), 2) ketamine (20 mg/kg IM; 12 animals), and 3) ketamine + dexmedetomidine (4 mg/kg + 25 ug/kg; 11 animals). Comparison of drugs were made in several areas including sedation, induction time, immobilization time, recovery time, indirect blood pressure, heart rate, respiratory rate, SpO2, blood gas, and cbc/chem. Induction (excellent/good/poor), muscular tension (absent, normal, increased), pedal withdrawal reflex (absent or delayed), palpebral reflex (absent, moderate, normal), and quality of recovery (smooth, satisfactory, rough) were measured by a blinded observer and a scorecard. Ketamine caused the quickest induction, alfaxalone caused the longest immobilization time (but with the poorest quality of induction), and ketamine + dexmedetomidine resulted in the shortest recovery time. There were significant differences in cardiac parameters between drug groups. Specific differences are best summarized in the figures below. Overall, the authors recommend ketamine + dexmedetomidine for brief sedation of healthy galagoes without heart disease due to fastest average recovery time and best quality of recovery.







QUESTION

1. What are some common names for Otolemur garnettii and what are they used for in research?

ANSWER

1. Northern greater galago, small-eared galago, Garnett’s greater galago, bush baby; vision and somatosensation

***Experimental Use***

**Whitehead et al. Biochemical Effects of Routine Gonadectomy on Blood of Domestic Ferrets (*Mustela putorius furo*), pp. 567-574**

Domain 3

Secondary Species: Ferrets (*Mustela putorius furo*)

SUMMARY: The domestic ferret is an important research model for the study of viral diseases, cystic fibrosis, cardiac disease, reproductive physiology, spinal cord injury, epilepsy, infectious disease, chronic obstructive pulmonary disease and chronic wasting disease of deer. Hematologic and serologic data is widely known, however, short term perturbations pertaining to surgical manipulations are poorly understood. Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR)-based techniques provide valuable insights, with NMR-based techniques preferred due to reliability and preservation of samples. In the current study, 14 intact ferrets underwent castration or ovariohysterectomy. Blood samples were collected in order to evaluate the difference between plasma biochemistry panels and one dimensional (1H) NMR whole-blood metabolomics analysis of presurgical baseline and post-op samples. The authors were able to conclude that the difference in magnitude of perturbations for plasma biochemistry samples was proportional to the duration of surgeries, which was considerably shorter for males as opposed to females. Forty-two metabolites and one drug residue was identified in the spectra of domestic ferrets via 1H-NMR spectroscopy. In addition, females had a greater propensity for changes between pre – and – postsurgical samples. In summary, this study highlighted the opportunity that 1H-NMR spectroscopy provides for characterizing biochemical perturbations in veterinary patients as well as the understanding of effects of surgery on physiology.

QUESTIONS

1.  What Family does the *Mustela putorius furo*belong to?

a.   Mustelidae

b.  Felidae

c.   Cyprinidae

d.  Bovidae

2.  The domestic ferret (*Mustela putorius furo*) is commonly used in research to study:

a.  Cystic fibrosis

b.  Reproductive physiology

c.   Chronic obstructive pulmonary disorder

d.   All of the above

3.  T/F: Gonadectomized female ferrets as opposed to male ferrets had a greater propensity for changes between pre – and – postsurgical blood samples via 1H-NMR spectroscopy.

ANSWERS

1.  a

2.   d

3.  True

***Case Study***

**Peterson and Berlin. Risk Assessment for Use of a Porcine Circovirus-Contaminated Reagent in a Barrier Maintained Rodent Colony, pp. 575-579**

Domain 3: Research

Primary Species: Mice (*Mus musculus*)

SUMMARY: Porcine Circovirus (PCV) is a major concern within the pork production industry. PCV2 causes postweaning multisystemic wasting syndrome and PCV3 has recently been considered the etiological agent for porcine dermatitis and nephropathy syndrome. PCV infections in pigs are endemic worldwide with evidence that other species, may carry or serve are reservoirs for the virus. Among various species, mice have been implicated in the spread of PCV among swine production farms. Additionally, PCV3 has recently been identified in commercially sourced BALB/c and ICR mice in China.

The presence of viral contaminants in pig tissues is a major concern among xenotransplantation developers. Evidence suggests that PCV can infect, persist, or propagate in a variety of cell cultures. The authors elected to monitor for PCV contamination from porcine-derived elastase (PPE) in their rodent barrier facility. Female Hsd:Athymic Nude-Foxn1nu (nude), BALB/cAnNHsd (BALB/c), and NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (SCID) mice were among the strains used for study; 7 of the 8 mice contained subcutaneous implanted tumors. Mice were treated with either PPE or recombinant human leukocyte elastase. PPE was obtained from 2 different suppliers, both of which tested positive for PCV2. One PPE sample tested positive for PCV3, the test results for the 2nd PPE was equivocal. Tissue samples harvested from the animals were negative for PCV2 and PCV3.

No evidence of rapid dissemination and amplification of virus to tissues beyond the lungs was found. The lungs of animals that received PCV-positive PPE appeared to be paler than normal with petechiae. Based on the results, the authors suggest that either the contaminant was not infectious, was rapidly cleared, did not have time to reach a detectable titer, or was below detection limits. They recommend submission of pulmonary and bronchiolar lymph nodes to determine the presence or absence of the virus.

QUESTIONS

1. Diagnosis of a PCV-related syndrome in pigs requires the presence of which?
   1. Compatible clinical signs
   2. Characteristic microscopic lesions
   3. PCV2 within lesions
   4. All of the above
2. PCV vaccines are commercially available for?
   1. PCV2a
   2. PCV2b
   3. PCV3
   4. A & B
   5. All of the above
3. Which breed is more susceptible to PCV lesions and disease?
   1. Duroc
   2. Landrace
   3. Gottingen
   4. Yorkshire

ANSWERS

1. d
2. a (the vaccine does not work for PCV2b or PCV3)
3. b